





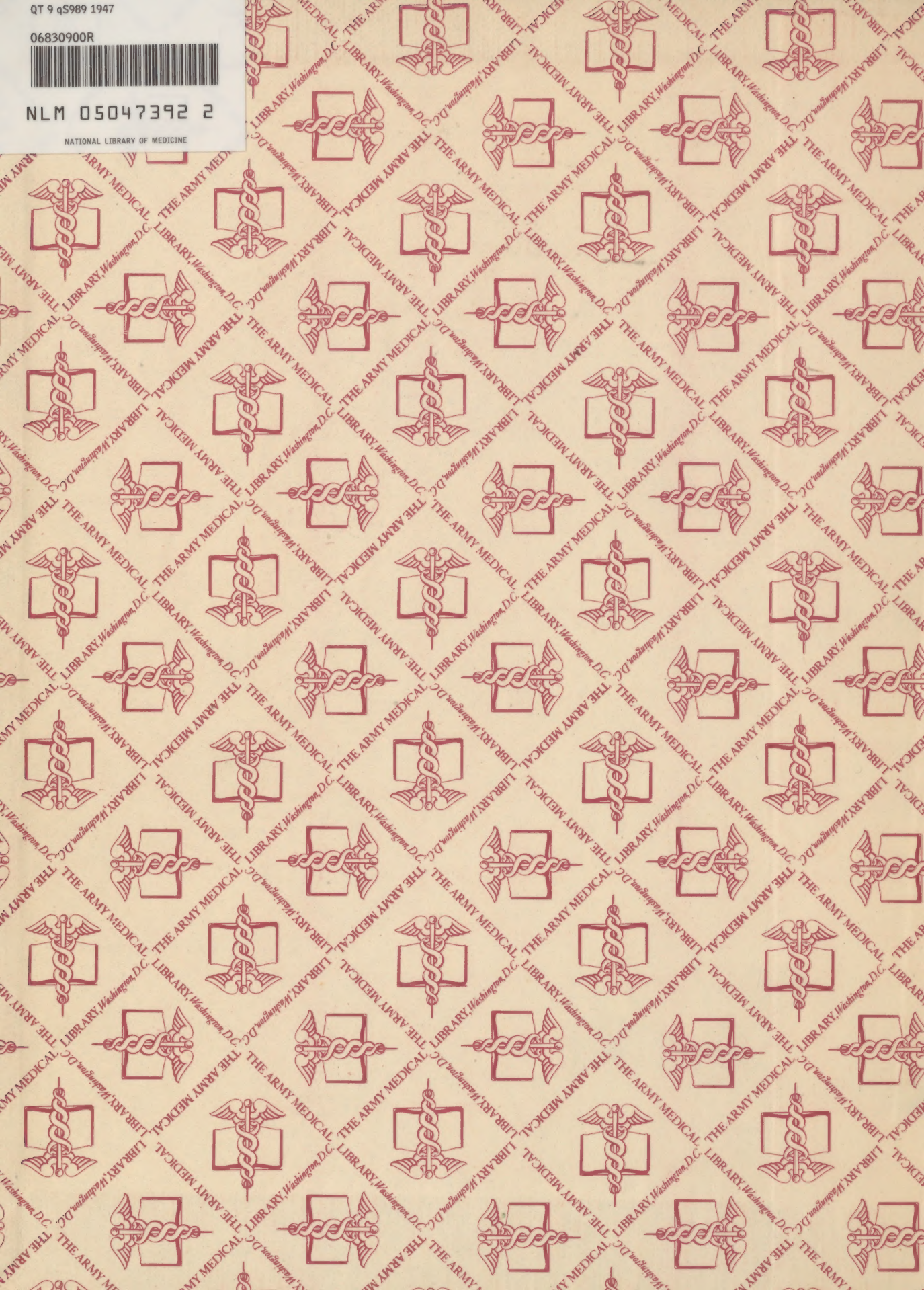
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**SYMPOSIUM**  
**ON**  
**MILITARY PHYSIOLOGY**, *Washington, 1947*

Under the Auspices of:

**THE MILITARY ESTABLISHMENT  
RESEARCH AND DEVELOPMENT BOARD**

**COMMITTEE ON GEOGRAPHICAL EXPLORATION  
PANEL ON PHYSIOLOGY**

**Washington 25, D. C.**

Prepared by:  
Department of the Army  
Chemical Corps  
Office of The Surgeon General

Approved: *Sidney Page*  
DIRECTOR  
COMMITTEE ON GEOGRAPHICAL  
EXPLORATION



STUDY

ON

MILITARY PHYSIOLOGY

Under the Auspices of

THE MILITARY ESTABLISHMENT

RESEARCH AND DEVELOPMENT

COMMITTEE ON RESEARCH AND DEVELOPMENT

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Prepared by  
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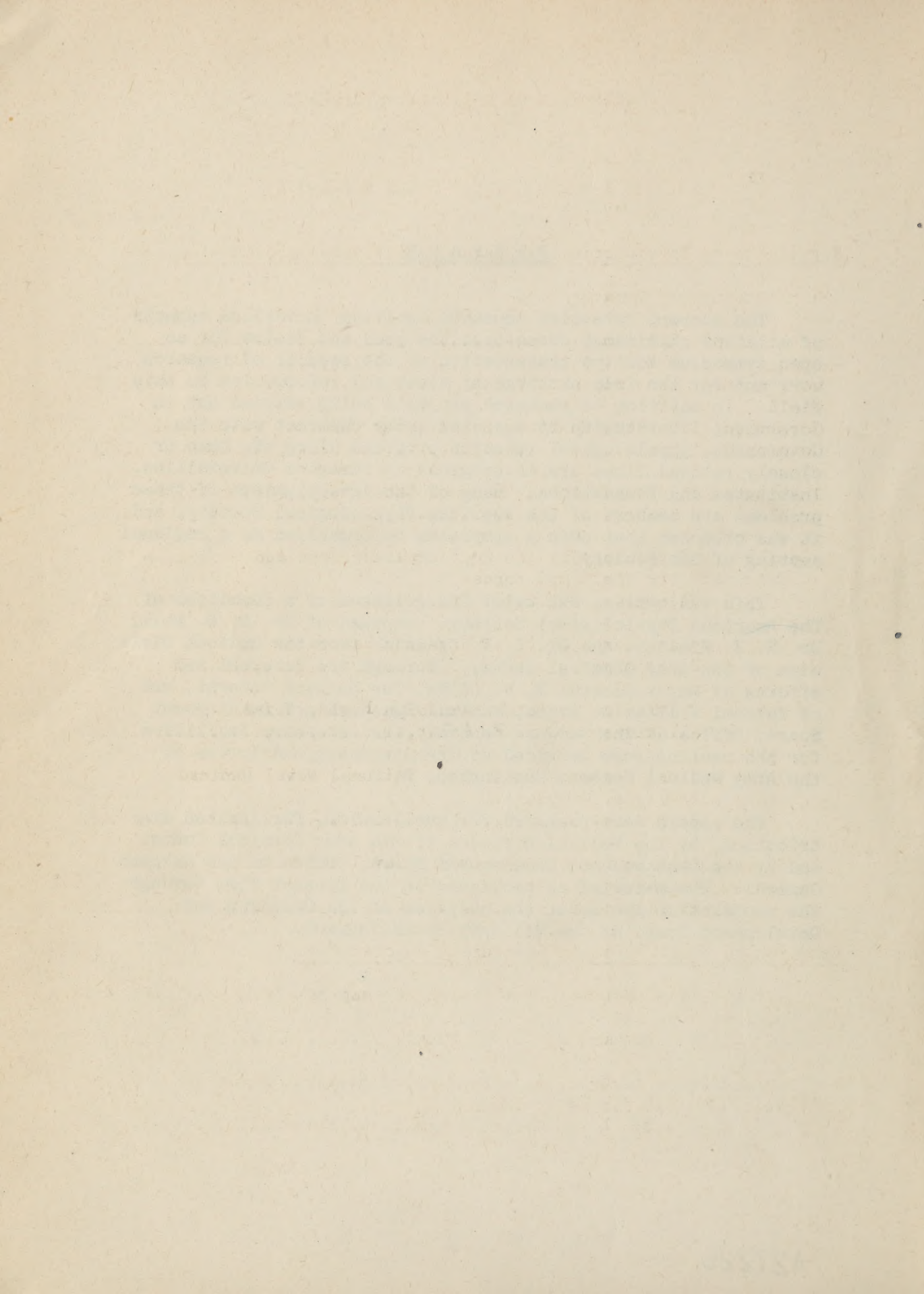
## P R E F A C E

The current intensive research activity in various aspects of military physiology occasioned the need and desire for an open symposium for the presentation of the results of research work and for the free exchange of views and information in this field. In addition to research projects being carried out in Government laboratories by agencies under contract with the Government, physiological research programs along the same or closely related lines are in progress in numerous Universities, Institutes and Foundations. Many of the investigators of these problems are members of the American Physiological Society, and it was proposed that such a symposium be organized as a regional meeting of the Society.

This was carried out under the guidance of a Committee of The American Physiological Society, composed of Dr. D. B. Dill, Dr. H. E. Himwich, and Dr. L. E. Chadwick from the Medical Division of the Army Chemical Center. Through the interest and efforts of Major General R. W. Bliss, The Surgeon General, and of Colonel William S. Stone, Medical Research and Development Board, Office of The Surgeon General, the necessary facilities for the meeting were provided at the Sternberg Auditorium of the Army Medical Center, Washington, D. C.

The papers were prepared for publication, for limited distribution, by the Medical Division of the Army Chemical Center and by the Research and Development Board, Office of the Surgeon General. The material is published in the present form through the cooperation and under the auspices of the Research and Development Board of the Military Establishment.





# SYMPOSIUM ON MILITARY PHYSIOLOGY

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## INTRODUCTORY REMARKS AT OPENING OF SYMPOSIUM

### . ON MILITARY PHYSIOLOGY

Colonel William S. Stone, MC, U. S. Army  
Medical Research and Development Board  
Office of the Surgeon General, Washington, D. C.

During July 1947 Dr. Bruce Dill of the Army Chemical Center suggested that the armed forces sponsor a symposium on physiological research being done by the Army, Navy, Air Force, U. S. Public Health Service, and on civilian research work being supported by these agencies. He also suggested that the symposium be held as a regional meeting of the American Physiological Society. That these suggestions were considered to be sound and in the best interest of all concerned as well as the advancement of science in all fields of physiology is confirmed by this occasion. We in the Medical Department of the Army welcome the opportunity to participate in these periodic presentations of physiological research being done by Governmental agencies and their civilian collaborators.

The solution of most research problems confronting the armed forces requires the closest teamwork between research workers in laboratories of the armed forces and investigators working in civilian institutions. Any step taken to improve this relationship is in the national interest, not only from the viewpoint of national defense but also for the greatest achievement possible in the fields of science involved.

Because of the necessity for independent thought and action in dealing with research there have always been considerable reservations held on the possibility of Governmental agencies' being able to provide ideal environment and backing for scientific research. We believe much is being done to eliminate these reservations both for basic as well as for applied research. Because many research problems facing the armed forces have a definite time limitation for their solution, it is sometimes necessary to encourage and emphasize work being done on these problems. However, we will always consider it essential to recognize the necessity for basic research and to back it with all the resources that can be made available.

It gives me great pleasure to open the Symposium on Military Physiology, and I hope that it is the forerunner of a long and close relationship between armed force and civilian research workers in all fields of physiology.





## INTRODUCTORY REMARKS

### ADDRESS OF WELCOME AT OPENING OF SYMPOSIUM

#### ON MILITARY PHYSIOLOGY

Major General R. W. Bliss  
The Surgeon General, U. S. Army

Welcome to the Army Medical Center! The Commanding General, his officers and men are anxious to do everything possible to make your stay a pleasant one.

I was most pleased to accept the invitation to open this regional meeting of the American Physiological Society and I wish to express my appreciation to our civilian scientific colleagues for their kind invitation to nine of our Army, Navy and Air Force laboratories to actively participate in the proceedings. My hope is that this meeting will be only the first of many similar ones, since the advancement of our medical knowledge, particularly in the basic sciences, is directly dependent upon the closest collaboration between the armed services and civilian research institutions. The interchange of information among investigative groups which occurs at such a meeting is both stimulating and informative.

It has often been noted, and is unfortunately true, that great strides in the advancement of medical science are only made during periods of conflict. Medical research has been a function of the federal government for more than half a century; but not until the war brought fresh and substantial sums of money to bear upon the nation's total scientific effort in this field did the government occupy a prominent position as an entrepreneur in medical science. Prior to our recent conflict neither the Army nor the Navy formally recognized research as a distinct function of their medical departments. The Army through the work of its faculty and students at this Army Medical School did make some contributions in the fields of bacteriology, immunology and preventive medicine. Extensive research was however impossible because of inadequacy of appropriated funds.

The war had a revolutionary effect upon medical research as a whole. Medicine and medical research received a tremendous impetus under which scientists produced phenomenal discoveries and advances. Scientists in colleges, universities and hospitals accepted a controlled and directed medical research program and they vigorously



cooperated with the government which became the leading financial supporter of research.

The war defined the problems in military medicine which demanded study and prompt solution. The leisurely independent pursuit of knowledge had to give way to organized hot pursuit of specific objectives. For example, chemicals to combat disease-bearing insects and animals, drugs to control traumatic and surgical infection; a quicker, safer cure for venereal disease; and more and better blood substitutes.

With the cessation of hostilities our nation as a whole is in a position to revert again to the time honored practice of leisurely and independent pursuit of knowledge. Basic research, or the theoretical analysis, exploration or experimentation directed to the extension of knowledge of the general principles governing natural or social phenomena, is perhaps the most difficult type of scientific endeavor. Basic problems require original thought of a high order by trained creative minds. The ultimate solution of fundamental problems or even the keys to their solution may not be found for many years. The best scientists may follow a number of fruitless leads before a key piece of information is finally discovered. Even this discovery may have no immediate application but it may later become the basis of full discovery, application and development. Only institutions with substantial resources can afford to support such research in a comprehensive manner. National policy and welfare, however, demand that an adequate balance be struck between expenditures for basic and applied research, since all major scientific advances are predicated upon new discoveries in basic fields.

The medical departments of the armed services are well aware of their responsibility in providing financial assistance and incentive for the undertaking of such fundamental observations and are devoting the major portion of their research funds to basic rather than developmental projects. The armed services furthermore, realize that they can ill afford to reduce their expenditures for research merely because of constantly declining military strength. We believe that as military forces in being decrease, funds for research and development must be increased, and we are jointly working toward the attainment of this objective.

In conclusion, may I re-emphasize that the Medical Departments of the Armed Services are fully aware of the part they must play in the advancement of scientific knowledge, are stressing research in the basic sciences as well as medicine and surgery, and are striving for the closest cooperation and understanding between themselves and all civilian scientific agencies. Furthermore, we believe that progress is only possible through continuous interchange of information among investigators.

## ADDRESS OF WELCOME AT OPENING OF SYMPOSIUM

### ON MILITARY PHYSIOLOGY

Major General Alden H. Waitt  
Chief, Chemical Corps, U. S. Army

I have read the program of this Symposium with much interest since it illustrates two principles now firmly established in the Chemical Corps, and which I have frequently stated:- Competent scientists are wedded to fundamental research, and fundamental research is vital to the success of a research and development organization. These principles first found acceptance in the Chemical Corps after World War I when several of our young officers were assigned as graduate students in the chemistry and chemical engineering departments of our leading universities. By the time of World War II, these officers, having reached positions of authority, were able to bring together a distinguished group of chemists and engineers whose research talents greatly influenced the course of the war.

The relation of the medical sciences to the program of the Chemical Corps underwent a tremendous transition during the war, due to the effective and harmonious working arrangements made between the Chemical Corps and the Medical Department. Colonel C. P. Rhoads, known to many of you as "Dusty" Rhoads, together with Colonel John R. Wood, were loaned by the Surgeon General. They were able to attract a brilliant staff of medical scientists in uniform, whose activities in the Medical Research Laboratory at Edgewood safeguarded our armed forces against the potential hazards of chemical weapons in the hands of our enemies, and helped arm our own troops in case chemical warfare became an actuality.

With the war over, Doctor Rhoads and nearly all his staff returned to their civilian posts. It remained for Colonel Wood, as Chief of the Medical Division at what we now call the Army Chemical Center, to build again almost from the beginning. This he has done with great skill and success. I am highly pleased and proud that he was able to attract Dr. Dill as Scientific Director of our Medical Division. It is a testimonial to the importance of the work we are doing. I am very proud of our research medical staff. The fact of this Symposium, initiated by physiologists at the Medical Division, is good evidence that the new civilian staff Colonel Wood has recruited is alert to their responsibilities.

Returning to the principle that scientists are wedded to research, I want to tell you in a few words how this works in the



Chemical Corps, and define my own views. I believe in the great importance of research and its high place in the National Defense System. When I say "research", I include in high priority basic research, research that cannot meet a deadline, research that may not show any immediate return. I believe that within the framework of the military organization, a strong and productive research program can flourish. I believe that having stated the objectives to be attained, the military must permit the scientist to carry out a program without interference. There has always been some criticism from civilian science of our military laboratories and some have held that an outstanding research organization could not be developed under military supervision. We are proving that this is not true.

Our scientists of Ph.D. caliber are encouraged to undertake original independent research within the broad field of Chemical Corps responsibilities. They are not placed under the onus of producing within six months or a year applicable results from such research. It is assumed that this policy will bear fruit in the form of fundamental discoveries of great importance to science; it is hoped and believed that some of these discoveries eventually can be applied by our engineers. In any case, it is certain that these scientists will grow in stature and will be strengthened in their loyalty to the Chemical Corps by this policy.

In return for the opportunity to conduct original and virtually untrammelled research, our scientists agree to bend their shoulder to the wheel and carry out "directed" investigations of immediate urgency, the nature and outcome of which frequently preclude publication. Few complain about this; if they do, a visit to some of our universities where heavy post-war teaching schedules cut into research opportunities has a salutary influence.

I am happy to join my good friend and colleague, General Bliss, in welcoming the scientists who have come here from many universities and from military laboratories reaching from the Quartermaster Climatic Research Laboratory in Massachusetts to the Air Forces School of Aviation Medicine in Texas. It is a splendid omen that ten of our leading universities are represented in tomorrow's program by physiologists whose research in the field of respiration is supported by military contracts; and that interspersed on the program are representatives of many other laboratories, both civilian and military. To my mind, this Symposium indicates that the close working relation between scientists in the service of their country, so firmly established during the war, will endure through these years of tumultuous peace.

# HIGHLIGHTS OF PHYSIOLOGICAL RESEARCH IN MILITARY

## LABORATORIES

### MEDICAL DIVISION, CHEMICAL CORPS

#### ARMY CHEMICAL CENTER, MARYLAND

D. B. Dill, Scientific Director

Why a symposium on military physiology? Probably many of you have asked this question and, possessing the scientist's full measure of curiosity, have come here to find the answer. As the program unfolds, those who have come will not be disappointed.

First of all, on behalf of the physiologists taking part in this meeting, I wish to thank General Bliss for his words of welcome, and his officers in the Research & Development Division and in the Army Medical Center who have helped with plans and have opened the doors of the Sternberg Auditorium to us. Secondly, it is a source of pride, particularly to us from the Army Chemical Center, that General Waitt has joined in the welcome. And, finally, your committee, consisting of Dr. Harold E. Himwich, Dr. Leigh Chadwick and myself, wish to thank the physiologists whose participation insures the success of this symposium.

The genesis of the idea for a symposium on military physiology came early last summer, when a number of physiologists came to the Army Chemical Center to discuss recent developments in biophysics. Those who attended considered the venture a success and many suggested "a return engagement". The idea took form during the summer and was presented in the fall to the other laboratories represented here. Their favorable reaction insured us of a nucleus of physiologists. Hence, in accordance with the constitution of the American Physiological Society, a regional meeting was organized and announced, and local members of the Society, their associates and students were invited to participate.

Returning to the original question, a brief review of the growth of the field of military physiology is in order. It is to the credit of Malcolm C. Grow, in his capacity as Air Surgeon, that he conceived in 1935 the idea of an Aero-Medical Laboratory in the research and engineering center of the Air Corps at Wright Field. This took shape during the next two years with Harry G. Armstrong in charge, assisted



by two physiologists, J.W. Heim and Ernest Pinson. Out of pioneering research in that laboratory grew Colonel Armstrong's solid reputation as an investigator, attested by his book, "Principles and Practice of Aviation Medicine"\*, and by his recent appointment as Commandant of the School of Aviation Medicine.

In the summer of 1940, Otis O. Benson, Jr., who was scheduled to succeed Armstrong at the Aero-Medical Laboratory, was assigned to temporary duty at the Harvard Fatigue Laboratory, which I was then directing. With the prospect of war, he proposed that I join him in building to a wartime scale the staff and the research program of the Aero-Medical Laboratory. Two of those who were invited to help accept-edaat once and subsequently played a major role in establishing the reputation held by the Aero-Medical Laboratory through the war as a leading center of research in military physiology. Happily, these two men, F. G. Hall of Duke University and E. J. Baldes of the Mayo Clinic, are on our program.

As war came closer and the National Research Council groups representing the various medical sciences were organized under what became the Committee on Medical Research, other military organizations recognized the need for physiological research. The laboratories established during this period need not be mentioned here, since most of them are represented on the program. However, because of my personal interest, particular reference is made to one that was considered at the beginning a bold venture, the QMC Climatic Research Laboratory. It is fair to say that its establishment in late 1942 came about as a result of the exploratory research during 1942 by the Fatigue Laboratory on Quartermaster cold weather clothing and equipment. Essential steps were taken by Paul A. Siple, famous geographer then on the Quartermaster Corps Research and Development staff and now having a related broadened responsibility with the Department of the Army, and by Georges F. Doriot, then in charge of the Quartermaster Corps Research and Development Branch. After an early formative period, John H. Talbott, a product of the Fatigue Laboratory, was placed in charge. Under his direction the Laboratory made a notable contribution to the problems confronting the Quartermaster Corps in clothing and equipping soldiers engaged in a global war. My relation to this laboratory dates from early 1943, --having completed my "tour of duty" at Wright Field I was assigned to the Quartermaster Corps Research and Development staff, with general supervision of field and laboratory research in the medical sciences conducted or sponsored by the Quartermaster Corps. It was a major responsibility during this period to guide our research in the light of reports by Quartermaster Corps observers on the reactions of soldiers to their rations, clothing, and equipment in the desert, in the tropics, and in the cold-wet conditions of North Africa, Italy, and Germany. Today, H. S. Belding, who played a major role in Fatigue Laboratory research during the war, will discuss the current physiological research program of the Quartermaster Corps Climatic Research Laboratory, which he now directs.

\*Published by Williams and Wilkins, Baltimore, 1939

My wartime experiences in the field of military physiology were climaxed by seven months in France and Germany during 1945, with an assignment as leader of a group of scientists and engineers asked by the Quartermaster Corps to seek for German developments that might be used in the Pacific war. After several months' contact with European soldiers and civilians, this group was convinced of the importance to our national security of a continuing close working relation between the soldier, the engineer, and the scientist.

Hence, when an invitation came a year ago to become Scientific Director of the Medical Division at the Army Chemical Center, I had little hesitation in accepting, nor have there been regrets for that decision. The Medical Division, under the skillful leadership of Colonel John R. Wood, loaned by the Medical Department to the Chemical Corps, had been brought through the difficult period of post-war readjustment and was well on the way to a peacetime "civilian" status. During the past year, the staff has been further strengthened and the post-war research program has been fully launched.

While the nature of the research program in most military laboratories prevents full presentation in an open meeting, this symposium proves that it is an accepted military policy to conduct some scientific research on "open" subjects. Only if this continues to be an accepted policy can first-rate investigators be attracted to military laboratories. It is my privilege to tell you something of such open research in the Medical Division that lies in the broad field of physiology. Details will be presented at subsequent sessions by several of my colleagues.

Physiology is one of the key sciences in the Medical Division. Granted that the first assessment of toxic agents involves a straightforward determination of lethality, of the quantity which will cause death in 50% of the animals tested, after that the mechanism of action must be revealed and therapeutic measures sought. Then one studies the physiological effects of sub-lethal amounts, the duration and intensity of effects on the nervous system, and on respiration, circulation, digestion, and metabolism. What limitations are placed on the capacity for work and what subtle influences may be exerted on the special senses and on the higher functions of the central nervous system? Again, what are the effects of protective measures on physiological functions and how can such effects, when deleterious, be minimized?

Such questions as these concern our Clinical Research Branch, headed by Harold E. Himwich, member of the Physiological Society and an authority on neurophysiology, respiration, and on the mechanisms of intermediary metabolism. His Physiology Section is led by Walter Fleischmann, whose co-workers, particularly Frank Craig and Francis Marzulli, are concerned with problems in environmental physiology.



Various items of the soldier's equipment developed by the Chemical Corps must be evaluated not only in relation to the protection they afford against toxic agents in all sorts of environments, but also in regard to their integration with various standard clothing assemblies appropriate to particular environmental conditions. The laboratory methods used include those developed during wartime - skin and rectal temperature, water balance, pulse rate, metabolism, working capacity, and subjective reactions. Such research will determine the adaptability of the soldier to the microclimates created within his protective clothing and, what is more important practically, will suggest improvements in protective equipment that will render its microclimate more equable. Animals as well as men may be involved; in a recent excursion into the field of comparative physiology, this group joined forces with the Quartermaster Corps Climatic Research Laboratory in determining the capacity of various domestic and laboratory animals to withstand cold. The most astonishing result was the survival of the fasting pigeon at  $-37^{\circ}$  C. for more than 24 hours; in fact, in 3 cases for 72 hours. Body temperature was successfully sustained near the normal until eventual sudden failure associated with a precipitous decline in body temperature.

A by-product of Chemical Corps research under investigation by some of our contractors is di-isopropyl fluorophosphate, commonly known as DFP. One of our contractors, David Nachmansohn, has explored the behavior of this substance in inhibiting cholinesterase and modifying nervous function. With the aid of DFP, he has examined the relation between the release and removal of acetylcholine in the surface membrane and the process of conduction in nerve and muscle. A second contractor, Kenneth E. Roeder, has used DFP to reveal the mechanism of conduction in the cord of the cockroach. Some of his conclusions cannot be reconciled with Nachmansohn's; further investigation is needed to elucidate fully the role of the acetylcholine - cholinesterase system in the various parts of the nervous system. The possible clinical usefulness of DFP in stimulating peristalsis have been described in a recent issue of the Johns Hopkins Bulletin by another contractor, A. M. Harvey and his associates. Its behavior in the body is being further explored in the Medical Division Laboratory by Harold E. Himwich and his associate, A. M. Freedman.

The effects of toxic agents on the circulation are of considerable importance to a full understanding of the mechanism of action. The technique of catheterizing the heart developed by Cournand and his associates has been taken up in the Physiology Section by one of our skillful young medical officers, Captain W. T. Goodale. He and his associates will describe to you, in a later session, their success in catheterizing the coronary sinus of dogs.

The field of respiration is of major concern to the Medical Division, since a common method of ingress of toxic agents is by way of the respiratory tract and the resulting injury may be localized there. Hence several contracts have been negotiated that cover research on one or another aspect of human respiration. Reports on two of these contracts will be made tomorrow by Drs. Ward Fowler and

James L. Whittenberger. Much research is under way in the Medical Division in this broad field. For example, Leigh Chadwick will describe for you his success in the study of respiration of insects in flight. His Section, Entomology, deals with fundamental problems of insect physiology, particularly as related to the mode of entry and mechanism of action of toxic agents on respiratory and nervous systems. Such research represents a useful by-product, since, in the study of toxic agents that come to the attention of the Medical Division, potential insecticides emerge. But this research is essential, quite apart from the occasional discovery of such by-products; knowledge of the action of toxic agents on insects, viewed in the light of their effects on other classes of animals, contributes to our understanding of their effects on man.

Many domestic animals are used in war and hence their reaction to toxic agents must be determined. Among these is the pigeon, already mentioned in connection with research in environmental physiology. Recently one of our veterinary officers, Captain R. W. Redding, has become interested in the accessory respiratory organs of the pigeon. He will present the results of this study at a later session.

The systematic evaluation of new toxic agents is carried out under the direction of William H. Chambers, Chief of our Toxicology Branch. This screening is done largely on rodents, and hence is likely to yield new rodenticides. Here again the physiological approach is useful as, for example, in determining the acceptability of potential and presently used rodenticides. The information obtained is of value not only in providing more effective pest control, but, also, in broadening our understanding of human and animal nutrition. The notable studies of one of our contractors, Curt Richter, on self-selection of diets by rats have rendered valuable service in these directions.

Much remains to be learned regarding gastric absorption and its relation to the physical and chemical structure of compounds, especially of those which might be used as contaminants of our food and water. Hence, about a year ago, the Toxicology Section initiated fundamental quantitative evaluations of the absorbability of various classes of compounds that might be used as chemical warfare agents. Such an approach is also employed in our Sanitary Chemistry Section, where techniques are developed for detecting poisons in food and water and for determining the actions of such poisons on appetite and digestion.

Physiologists in wartime were sometimes asked, "Are present expenditures in the field of military physiology sound?" This type of question is by scientists disliked. It is a legend that Faraday had an answer to such a question appropriate to the occasion. When a



housewife once doubted the usefulness of his galvanic cell, he replied, "Madam, of what use is your baby?" On another occasion he is said to have remarked to a member of Parliament, who also questioned the usefulness of the galvanic cell, "Wait ten years and you can tax it." It is the nature of scientific research that it is completely impossible to tell in advance that a given research project will turn out successfully or that, even if scientifically successful, its outcome will immediately enable management to make more money or generals to turn the tide of battle. From time to time, however, it is fair to view in retrospect the cost of a research program and to estimate its immediate usefulness. In doing so, one should not inquire too closely about every element in the program, bearing in mind that some of the seemingly trivial scientific advances of today will become crucial to the major scientific achievements of tomorrow. It is not the purpose of this paper to calculate the costs of the wartime investigations of physiologists. Certainly the wartime costs were small, for of 1,000 professional physiologists in the United States, only a minority were engaged directly in war research. It is stated confidently, however, that the funds physiologists used in basic research and in cooperation with engineers in applying such basic research to their development programs were trivial in amount when contrasted with their impact on the course of the war. If one considers, for example, those stresses associated with high altitude flight, untold savings in men and materiel came about through basic research that made possible the development of our present equipment for supplying oxygen to air crews. Again, one considers the military losses through the effects on man of cold and heat, and the small fraction of the country's potential physiological research talent that worked in this field, the cost of physiological research on such stresses is incredibly small.

What types of research should be conducted in military laboratories? Should we venture into the field of pure research, or should our program be limited to applied research? The answer is clear; it is impossible to differentiate sharply between pure and applied research and therefore out of the question to define precisely the type of research to be done in military laboratories. At the extremes, the difference is obvious, but there is a great middle ground, where distinction is impossible. Investigators in military laboratories should be given much freedom for work in this area; without it, their minds will become dulled and their originality, ingenuity, and enthusiasm - all essential to research activity - will be stifled.

In conclusion, it is hoped that out of this first symposium will develop a better understanding and a closer working relation between the staffs of military laboratories represented here and between military and civilian physiologists. We all have a vital stake in national security. It devolves particularly on scientists in the various Government laboratories to work with full mutual understanding among themselves and with free exchange of information in so far as security permits. To overlap to some extent facilities and research undertakings is understandable and desirable, so long as this is done in a spirit of friendly rivalry rather than in a spirit of jealous secrecy.

## MEDICAL DEPARTMENT FIELD RESEARCH LABORATORY

FORT KNOX, KENTUCKY

R. G. Daggs, Director of Research

The Medical Department Field Research Laboratory, as it now exists, is an outgrowth of the Armored Medical Research Laboratory. The Armored Medical Research Laboratory was established at Fort Knox in September 1942, and was operated under the control and supervision of the Commanding General, Army Ground Forces. Its designated mission was that of studying and solving physiological problems arising from service with armored vehicles. In February 1944, the jurisdiction of the Armored Medical Research Laboratory was transferred from the Army Ground Forces to the Army Service Forces and the laboratory was designated as a Class IV installation under the control of the Office of the Surgeon General. The mission of the laboratory was then broadened to cover physiological and psychological problems of military personnel in general with special reference to equipment, environment and military tasks.

The major projects carried out by Armored Medical Research Laboratory were as follows:

1. Cold Weather Operations
2. High Temperature in Tanks
3. Toxic Gases in Armored Vehicles
4. Dust Exposure in Armored Vehicles
5. Crew Fatigue Research
6. Vision in Tanks
7. Methods of Pre-selection of Armored Force Personnel
8. Anthropometric Measurements of Armored Force Personnel
9. Gunnery Problems

Over 175 reports were submitted on these major projects. Practically all the work done during the war was of an applied nature. Necessity demanded quick answers to practical problems. Although applied physiology was the major field of endeavor, testing and developmental work in chemistry, physics and engineering was accomplished. The Armored Medical Research Laboratory scientific staff was recruited from civilian research institutions. The scientists were granted commissions in the Army of the United States and were stationed at the laboratory for the duration of the war.

Early in 1946, even though the Armored Medical Research Laboratory had completed its wartime mission and its military scientific



staff had returned to civilian life, it was realized by the Surgeon General's Office and the Research and Development Board that this laboratory, which had contributed so much valuable information during the war, should continue its work during peacetime. It was decided to staff it with a combination of civilian and military scientists and to change its mission to include long range basic studies in various related medical fields but to maintain major emphasis upon physiology.

In April 1946, efforts were begun to reorganize the laboratory and recruit a permanent scientific staff. On 1 April 1947 the laboratory name was officially changed to Medical Department Field Research Laboratory.

### Present Organization

It is not within the scope of this presentation to discuss the administrative branches of the laboratory. It will suffice to state that these activities are mainly under the jurisdiction of the military staff. It is also pertinent to point out that a mixed civilian-military staff, in the experience of this laboratory to date, works smoothly and efficiently. The research branch, which also includes both civilian and military members, is mainly under the jurisdiction of the civilian scientific staff.

The research branch is made up of three major sections: Physiology, Biochemistry and Biophysics. Functional groups or research teams are set up under each section as requirements demand. At present the Physiology Section includes the following functional groups:

Cardiovascular Physiology	Renal Physiology
Field Metabolic Studies	Neurophysiology
Clinical Physiological Research	Hypothermia Studies
Psychophysiological Studies	Testing

Biochemistry includes the following functional groups:

Fat Metabolism Studies	Protein Chemistry
Carbohydrate Metabolism Studies	Radioactive Isotope Studies

Biophysics includes the following functional groups:

Radiation Studies	X-ray Studies
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The present staff includes the following civilian scientists who head sections and research groups:

R. G. Daggs, Director of Research	E. A. Blair, Chief Physiologist
D. E. Gregg, Chief Research Physician	H. F. Jensen, Chief Biochemist
H. F. Kuppenheim, Chief Biophysicist	R. W. Clarke, Physiologist
A. W. Carpenter, Chief Engineer	G. W. Molnar, Physiologist
K. P. McConnell, Biochemist	E. D. Palmes, Physiologist

Research Officers (MC and MSC), civilian technicians, and enlisted technicians comprise the remaining personnel of the research groups.

Early in 1946, at the time of the reorganization of the laboratory, authority was granted the laboratory to set up sub-projects, under any of the following authorized broad project headings:

1. Study of the Physiological Effects of Cold.
2. Study of Physiological Effects of High Temperature.
3. Study of Body Measurements as They Affect Physiological Efficiency.
4. Study of Body Reactions and Requirements under Varied Environmental and Climatic Conditions.
5. Studies of Fatigue in Relation to Military Tasks.
6. Studies of Physiological and Psychological Problems of Military Personnel in Relation to Equipment, Environment and Military Tasks.

During the past year the following 16 sub-projects have been completed:

1. Observations on the Relation of Height of Heel and Support in Arch of Shoes to Foot Physiology in Marching Troops:

There was no essential difference between the effect of the control and experimental shoes on the foot health of the marching troops as evidenced by the frequency, type, duration, distribution, time of onset and severity of the clinical lesions present.

What more critical tests, involving dynamic or static changes in bones and their relationship to various marching stresses, will accomplish remains to be determined.

2. Photoplanator:

A laboratory model of the non-parallaxtic photoplanator has been developed using a high intensity source approximately 0.006 inches in diameter. The assembly shows something less than 0.0025 of an inch of parallaxtic deviation in dimensions up to 3 inches.

3. A Method of Human Calorimetry:

A method has been devised which can measure each component of the thermal balance continuously. Rapidly changing and high rates of evaporation and metabolism can be determined without difficulty.



4. An Improved Mounting for Thermocouples for the Measurement of the Surface Temperature of the Body:

The improved mounting on open mesh wire can be attached to a subject rapidly and easily and always remains firmly in place despite heavy sweating and muscular movement. Radiometric and thermocouple reading compare very favorably in a variety of ambient temperatures.

5. Thermal Regulation During Fever:

Three physiological mechanisms are principally concerned: peripheral blood flow, sweat secretion and muscular activity. These mechanisms operate in certain patterns to produce fever depending on the environment and the strength of the pyrogenic stimulus.

6. An Apparatus and Method for the Continuous Measurement of Evaporative Water Loss from Human Subjects:

Modification of an Infra-Red Gas Analyzer to record differences in water vapor content of inlet and outlet air permits continuous recording of evaporative water loss.

7. Thermal Regulation During Early Acclimatization to Work in a Hot Dry Environment:

The principal thermal adjustment of acclimatization was the development of a higher rate of sweat secretion. The added cooling by evaporation lowered the skin temperature and improved the internal thermal gradient permitting the critical deep tissue temperature to be maintained at a nearly normal value.

8. A Critique of Physical Fitness Tests:

None of the standard tests permits a satisfactory discrimination between degrees of individual fitness. A battery of fitness tests is a better measure than a single test. They are fairly satisfactory as gross measures of fitness and permit comparison of groups.

9. Silica Content of Dust from Tank Ranges:

The content of silica of a damaging character in air borne dust at Fort Knox is regarded as high but the data do not permit conclusions as to whether or not inhalation of silica particles of such concentration and particle size distribution constitutes a medical menace to operating armored personnel.

10. Efficiency of Signal Corps Operators in Extreme Cold:

Present types of adequate protective clothing produced by

its bulkiness a loss of efficiency that completely overshadowed the loss of efficiency due to cold, per se, during short exposure times.

11. Plastic Ear Mold for Communications Equipment:

Plastic ear molds are individually made and fitted. They are not interchangeable. Temporary hearing loss from field radio communication was less with the ear mold and there was a greater signal to noise ratio. The molds are capable of use in extreme temperatures and produce no injury to the ear.

12. Application of the Infra-Red Gas Analyzer to the Study of Human Energy Metabolism:

The Infra-Red Gas Analyzer equipped with an adequate pumping system for controlled air flow, has been adapted to metabolic work (CO<sub>2</sub> determination) and found satisfactory, both in the laboratory and in the field.

13. Observations - Frigid and Williwaw: Restricted report.

14. Preliminary Observations on Physiological Nutritional and Psychological Problems in Extreme Cold - Ft. Churchill, Winter 1946-47.

15. Physiological Observations, Task Force Furnace: Restricted report.

16. Effects of the Cold Pressor Test on Glomerular Filtration and Effective Renal Plasma Flow:

As a result of cold stimuli, the glomerular filtration rate and effective renal plasma flow decreased 14% and 21%, respectively.

The following sub projects are now in progress at the laboratory:

1. Effects of Hypothermia on Vitamin-A and Fat Metabolism in the Rat.
2. Studies of the Effects of Subnormal Temperatures on the Metabolism of Various Phosphorous Compounds in Rats.
3. Development of an X-ray Stereopanoramograph.
4. Development of a Cineroentgenograph.



5. Renal Circulation and Excretion as Affected by Exercise.
6. Relation of Dietary, Metabolic and Mechanical Factors to Atherosclerotic Lesions in the Rat.
7. Aortic Factor in Hypertension.
8. Observations on the Accuracy of the Electromagnetic Flow Meter.
9. A Possible Humoral Factor in Human Thermal Regulation.
10. Analysis of Cortical Components Involved in Reflex Pupillary Dilatation to Clarify the Etiology of Anisocoria Resulting from Head Trauma.
11. Development of a Photoelectric Polarimeter with Variable Electric Field.
12. Development of a Self-Contained Portable Apparatus for Study of Human Energy Metabolism.
13. Spectrophotometric Studies of the Effect of Ultraviolet Radiation on the Skin.
14. Studies of Physiological Problems under Field Conditions in Extreme Cold.
15. Development of a Polarographic Flow Meter.

MILITARY PHYSIOLOGY IN THE QUARTERMASTER FOOD  
AND CONTAINER INSTITUTE FOR THE ARMED FORCES

George Gelman, Technical Director, Quartermaster  
Food and Container Institute for the Armed Forces

We wish to take this opportunity to acknowledge with sincere appreciation not only Dr. D. B. Dill's efforts in making this symposium possible but in addition the scientific prestige that he brings to the military forces. Dr. Dill's accomplishments in physiology as Director of the Harvard Fatigue Laboratory are well known. Significant and fortunate for us have been his activities at the Aero-Medical Laboratory of the Air Materiel Command, Army Air Forces, the Research and Development Branch, the Office of The Quartermaster General, and now as Scientific Director, Medical Division, Army Chemical Center. In addition to his many duties, Dr. Dill is a member of the Scientific Advisory Board of our Institute.

In order to provide a setting for our activities in military physiology, it is appropriate to tell you about the Institute itself in terms of location, personnel, facilities, mission, and operations.

The Quartermaster Food and Container Institute for the Armed Forces is located in Chicago, Illinois, the heart of the food and container industries of the country. It is particularly fortunate in having for its next-door neighbor the Medical Nutrition Laboratory of The Surgeon General's Office. This close proximity permits an excellent cooperative approach to problems of mutual interest to The Surgeon General and the Quartermaster General.

The total personnel strength of the Institute is 196, of which 32 are military and 164 are civilian. Scientific personnel total 116.

The Institute has for its primary responsibility the research and development of foods, rations, containers for Quartermaster supplies, and advanced subsistence training. Designed and established during World War II, the Institute is one of the largest and most modernly equipped installation of its kind in the world. It has for its peacetime functions:

a. Servicing the present technical food and container needs of the Armed Forces.

b. Developing foods and containers that would be required in the event of a national emergency.



c. Administering a food research and development program in cooperation with approximately 500 industrial and university laboratories.

d. Training Armed Forces and allied officers for key technical subsistence assignments.

The Quartermaster Corps has consolidated all of its food and container research in the Institute, and in addition the Institute serves the Army Ground Forces, United States Army Air Forces, and provides information to the Department of the Navy. It is important to point out that the activities of the Institute are coordinated not only with the Armed Forces but also with the various Government and industry research agencies whose activities are of mutual interest.

The Institute is responsible for the preparation of all food ration and Quartermaster Corps packaging specifications. Through its membership on the Federal Specifications Board and the Joint Army-Navy Specifications Board, the Institute is able to reflect the latest information resulting from research and development.

The Quartermaster Food and Container Institute for the Armed Forces Food Research and Development Program arises from a need for information necessary to develop rations required for the constantly changing military science of tactics and logistics, and to service the present tactical ration needs of the Army.

Military foods and rations are distinguished from civilian products by the necessity of special attributes of stability, acceptability, nutritional adequacy, and utility. Such essential military problems as the design of arctic rations, survival rations for ditched personnel from planes and Ground Force troops, in-flight feeding rations for use in new types of planes, and adaptation and utilization of frozen pre-cooked foods depend for development or improvement on the fundamental investigations that are now under way.

The Quartermaster Corps has already replaced almost every World War II ration with new operational rations. It must be emphasized, however, that the military characteristics of foods and rations are only partially met in these new developments. The Institute's fundamental research program is pointed toward filling the gaps in our present understanding of the science of food and nutrition. The program consists of seven major areas:

1. Food Research Survey and Planning
2. Product Development
3. Ration Development
4. Container Research and Development
5. Ration Stability
6. Ration Acceptability
7. Ration Nutrition

## Food Research Survey and Planning

To ascertain the number, nature, and particular interests of the major food research programs of the United States, we are conducting a comprehensive survey of those now operative in the colleges, universities, government experiment stations, industrial laboratories, and foundations throughout the nation by a grant to the National Research Council. The value of such a survey to research planning is manifold. It should reveal the programs that are of particular significance to military feeding and ration design, it should uncover areas of research that are not at present being adequately investigated, and it should provide an inventory of facilities available for research on food composition, deterioration of foodstuffs as affected by chemical, microbiological and physical factors, and similar problems of concern to the Armed Forces. The overall value of the survey to the mobilization of food research facilities and personnel in the event of an emergency is apparent. The program will insure that food research information on a nation-wide basis is not only available to the Army but to the entire country. Unwarranted duplication of research effort will be minimized. This will be a continuing project.

A survey is being made of the raw material and chemical requirements of the food processing industries, with particular reference to those materials that must be imported or are not readily available. This information will be used for preparing stockpiling estimates and as a basis for the development of substitute items to replace critical materials.

### Product Development

Product development is concerned with the improvement of texture, flavor, appearance, acceptability, and storage qualities of existing food products used in military feeding and also with the development of new products when required. The results of basic research are studied and applied to processing animal, fruit, dairy, cereal, and general products in order to produce ration components that are utilizable in the field, acceptable to the troops, stable under storage, and nutritionally adequate. The results of this investigation are reflected in military specifications which are the basis of Armed Forces procurement. Much of the investigation is accomplished in collaboration with industrial organizations throughout the United States. This will be a long range continuing program constantly reflecting the latest advances in science and technology to Army Foods and rations.

### Ration Development

Modern warfare presents many situations where kitchen facilities being unavailable, soldiers are forced to consume their food singly or in groups. Since commercial foods do not ordinarily meet the special requirements of this situation, operational rations must be provided.



These ration assemblies of different products must be designed for specific tactical or military situations. Although one of the basic considerations is ease and convenience of field usage, each ration must be related to a well-recognized military situation. Thus, there are rations suited for use in combat, for survival after isolation from the main action, for use in the arctic, for in-flight feeding, etc. Each of the components of these rations must be sufficiently stable to withstand long storage and to retain during that time their palatability and nutritional adequacy. This will be a long range continuing program as necessitated by the changing military sciences of tactics and logistics.

### Container Research and Development

The object of container research is to insure that products are adequately protected from the hazards of handling and adverse conditions of storage, from the point of origin to the point of use. To accomplish this objective, it is necessary to conduct basic research on container materials with special attention to their strength and their resistance to moisture, insect, mold and bacterial damage. The ultimate object is to design containers that will afford maximum protection to their contents. The many variables involved in designing containers for the specific use to which they must be put require the development of new methods of testing, special techniques for predicting the serviceability and performance of a given container, and a thorough acquaintance with the physical and chemical properties of container materials. This will be a long range continuing program to insure that Quartermaster supplies will be adequately protected from a packing, packaging, and crating standpoint, against all types of climate, storage and handling.

### Ration Stability

Food for military use must be shipped long distances and handled and stored under adverse conditions. Foods for civilian use, on the contrary, are handled and stored under the most favorable conditions. In consequence, research into methods of insuring the stability of foods under the extreme climatic and environmental conditions dictated by military necessity is required. The three broad types of food deterioration that must be controlled are those resulting from chemical and histological changes and from microbiological growth. The problems of greatest concern are the oxidation of fats and fat-like substances, the discoloration of processed foods, known as the browning reaction, the degradation of starch and of pectin, the growth of micro-organisms, and enzymatic and histological changes that may affect foods. Many of these deteriorative changes become greatly accelerated during storage for long periods, particularly at elevated temperatures. The control or prevention of deterioration must begin with basic studies on the raw materials used and the processing methods employed by the manufacturer. Samples must be analyzed during various stages of storage to determine the effect on deterioration. The ultimate objective

of the investigations is to minimize or prevent deterioration. This will be a continuing project until it is learned how to process foods in such a manner that they will remain stable for at least two years.

### Ration Acceptability

Acceptability of foods and rations is of utmost importance in military feeding programs because it involves directly the health and morale of troops. Troops have no opportunity to select the foods they desire and their dietary habits are heterogeneous since the individuals composing military units come from all parts of the country. Superimposed on the difficulty of predicting feeding programs having optimum acceptability are environmental factors such as:

- a. Feeding under assault conditions
- b. Feeding under combat conditions
- c. Feeding under survival conditions
- d. Feeding under rear area support conditions
- e. Effects of climate
- f. Effects of psychological factors such as:

- (1) Anxiety
- (2) Tension
- (3) Fear
- (4) Monotony

g. Food habits, such as food patterns, preferred method of preparation, optimum frequency of serving familiar foods, etc.

Food acceptance research involves many of the biological, physical, and social sciences. The integration and application of these sciences to civilian feeding has had very little attention. Almost no information is available for military use, and it is of utmost importance to investigate the factors involved. This too is a long range continuing program.

### Ration Nutrition

Nutritional and physiological adequacy are fundamental factors underlying the design of rations. Entire areas in the science of nutrition and physiology require investigation to determine the effect of prolonged storage on nutrient retention and the development of toxic compounds. Little information is available of the nutritional requirements and physiological mechanisms imposed by the extreme conditions of modern warfare. The nutritional needs of troops under assault, air and ground combat, and even the feeding requirements of troops prior



to assault are being investigated. The physiological mechanism of food metabolism and the nutritional requirements under conditions of reduced caloric and water intake necessitate fundamental investigations in animal and human physiology. More rapid methods for evaluating future rations to insure nutritional and physiological adequacy require development since constantly changing logistics and tactics necessitate frequent modifications in ration design. The efficiency, health and preservation of life itself are directly related to the development of fundamental areas in ration nutrition research. Private or public research agencies are not ordinarily motivated to undertake this type of investigation without support. This will be a long range continuing program.

Of particular interest to this symposium is the Institute's research program pointed toward nutritional requirements and psychophysiological aspects of hunger and thirst. This program is patterned to provide a basis for designing rations that may be used under peculiar military stress conditions such as:

- a. Environment
- b. Noxious stimuli
- c. Work output
- d. Limited food and water

It is important to determine better methods for measuring nutritional adequacy, mechanisms of food utilization under both normal and abnormal stress conditions, and the mechanisms controlling food and water intake under both normal and abnormal conditions.

Stress may arise from a number of causes: environment - with extreme hot and cold climates; noxious stimuli - such as noises, frustrations; demand for increased work output, resulting in necessarily increased caloric intake. This increased work output may at times coincide with the restricted food intake. The restricted food intake produces certain physiological changes which place stress on the individual, both physically and psychologically. Not the least of the psychological stresses resulting from low food intake is the phenomena of appetite, the non-gratification of which causes mental distress.

The development of rations which would be adequate in each of the above situations demands an understanding of the basic physiological processes and the manner in which they are changed by stress. Unfortunately, only fragmentary data on this subject relates to the normal organism in normal circumstances.

The various phases of the problem have been handled by a wide variety of institutions throughout the country. While results are still incomplete, considerable progress has been made toward a better understanding of the changed needs of individuals under abnormal conditions.

In order to find out what foods were essential for life under cold temperatures, the Emory Thurston Laboratories raised rats at zero degrees Centigrade. These animals gained much less weight than rats maintained at 20° Centigrade on the same ration. Supplementation of the basic diet with various substances was carried out in order to determine if this stress produced increased needs for various vitamins. Results showed this to be negative in the case of all the known vitamins. However, rats raised on a basic diet supplemented by either liver or yeast gained more weight than animals who received the Vitamin B complex in purified form. This seems to warrant the conclusion that both yeasts and liver contain an essential nutrient, the needs for which are increased under cold room conditions. They also found that massive doses of Vitamin B fed either ad lib or under the stress of reduced caloric intake produce no untoward effect.

Work has been done on the effects of high temperatures on experimental animals. Brobeck of Yale has found that the amount of food ingested by rats decreases with the rise in temperature. At 95° F. rats eat little, drink a great deal of water, and, if maintained at this temperature, lose weight. These results may not be significant due to species difference, as the rat has a poorer heat exchange mechanism than the human.

It is well known that under the stress of excitement the desire for food disappears. This brings up the question of whether or not the exposure to noxious stimuli alters the physiological needs of the individual. In experiments carried out on rats by Patton at Pittsburgh, the food consumption and the free choice selection of food was determined first under normal conditions and then under conditions of stress. Stress was arbitrarily provided by a special apparatus which subjected the rats successively to an electric shock, the noise of a buzzer, and a blast of air. These three stimuli were provided at intervals of one to three minutes and were maintained for a period of 14 hours. Later work increased the exposure time to 24 hours. Rats were also taught to solve a problem. After they had mastered this, the problem was rendered insoluble by changing one of the physical factors. The frustration induced by these two methods resulted in abnormal behavior while the rats were under strain, but did not alter the pattern of their food selection or intake. The animals also showed the same ability to learn a maze, both before and after exposure to the above conditions. Possibly the rat is not high enough in the animal scale to show effects of nervous instability brought on by psychological adverse stimuli. Starvation, as a form of stress, also produced no observable changes in the rats' ability to select a diet or adapt to situations.

The physical work necessitated by military activity is often in excess of the same individual's output under normal conditions. This requires an increase in the caloric intake to maintain the individual. In this connection it was desirable to ascertain what effect temperature had upon caloric output. Dill, Gray and others have determined the effect of clothing separately from the effect of temperature on the caloric output necessary for a given piece of work. Three different uniforms, Arctic, Temperate, and Tropic, were tested at -15° F.,



60° F., and 93° F. This work was carried out on normal college students in good physical condition and maintained on a high calory diet, after an adjustment period in which the students were standardized on work output by learning to perform the task in cadence to a metronome. They were also accustomed to breathing into a bag to accustom them to a metabolic determination to be made during the test of the uniforms. The test of the Arctic uniform at 93° was omitted as during a test run of the temperate uniform at this temperature the individuals showed an increase of three to four degrees in rectal temperature and a terminal heartbeat of 178. The concurrent fatigue was so severe as to preclude any more rigorous test. The remaining tests were carried out without incident. The results show that the caloric output for a standard piece of work at constant temperatures increases about 5% when clothing is changed from desert to temperate, and an additional 5% at the change from temperate to Arctic clothes. Caloric output for each uniform decreased about 2% as the temperature was raised from 15 to 60° F., and an additional 2% from 60° to 90°. This seems to establish the fact that the clothing so inhibits action that the work performed is increased. Tests run on nude men pedaling against a fixed resistance showed no increased energy expenditure as temperature was raised from 0 to 50° C. In comparison with this, Harve and Berryman have observed no seasonal variation in the voluntary food consumption of soldiers within the Continental United States.

Early work indicated that rats allowed free choice selected a diet adequate for their physical well-being. Patton at Pittsburgh, however, discovered that rats, when allowed to eat ad lib of salts, proteins, carbohydrates, and fat, did not always eat sufficient protein to survive. The remaining rats were about equally divided between animals who consumed most of their calories as carbohydrate and others who preferred their calories as fats. From this work he concluded that the appetites for minerals and calories were specific, but those for the other essentials of diet were not.

Further work by Shills and Goldwater at Columbia University indicates that there is little relation to the physical needs and free selection. Habit is an essential factor in the choice of food. This was determined by raising animals on complete diets of a particular flavor. This diet was later removed, and a non-flavor complete diet offered in conjunction with an incomplete diet flavored like the previous complete one. The animals unhesitatingly chose the incomplete flavored diet.

Some very interesting work was carried out by Hollander at Mount Sinai Hospital on a human subject with a cardiac occlusion of the esophagus. This subject received daily feedings by jejunal fistula. Although his intake was adequate, he still showed a desire for food by mouth, which was promptly regurgitated. His preference for food by mouth was almost entirely for carbohydrates. When liver substance was added to his diet, his voluntary consumption reached the level of 10,000 to 11,000 calories. At this time his actual food intake was around 3,000 calories. His voluntary intake continued high after the liver substance had been removed from the diet and did not begin to

drop until the caloric intake had been increased to 4,000 per day for two weeks. At this level of caloric intake his voluntary intake and desire for food gradually abated. Evidently liver substance increases the needs of the organisms for food by a complex physiological process. In dogs intravenous injections of amino acids reduce the voluntary food intake far more effectively than did intravenous glucose. (Thomas at Jefferson Medical).

Goetzl at the Permanente Foundation suspected the correlation between the sense of smell and appetite. The reduction in the desire for food is a phenomenon most of us have experienced during a cold. During this work, subjects were standardized by means of an apparatus which could accurately measure the amount of vapor of the substance being examined. It was found that the threshold of smell decreased, i.e., a smaller amount produced the same sensation before meals. After meals the reverse was true, more vapor being required for the subject to perceive the odor. Evidently satiety decreased both appetite and the ability to perceive odors. Amphetamine has been used as a drug to reduce appetite for persons desiring to lose weight. This drug was given orally to the subjects prior to luncheon, and, on a subsequent trial, concurrent with the noon meal. The caloric intake of both days was determined as well as the acuity of smell both before and after meals. The perception of odor, as was expected, decreased after luncheon, including the amphetamine, and surprisingly, remained low for the entire afternoon. The morning administration of the drug not only raised the threshold of smell, but also "killed" the subject's appetite for food. They noted a feeling of fullness, which persisted well into the afternoon, although their caloric intake was considerably less than normal. Results opposite to those of the above experiments were obtained when bitters were substituted for amphetamine.

The only other drug which has an adverse effect on appetite and is sufficiently mild for possible use is gossypol, obtained from cottonseed oil. Dr. Zucker at Columbia University has made a fairly extensive study of this substance. The addition of a small quantity to the diet reduced the food intake of rats, with no unusual symptoms. After this agent was removed from the diet, the rats ate normally and regained lost weight. No evidence of detrimental action was observed. In order to determine what the action might be, rats were autopsied at intervals after ingestion of gossypol. The stomachs were filled and the upper intestine empty. As the time interval increased, less food was found in the stomachs of the gossypol-fed rats, but in no case as little as was found in normal rats on a non-supplemented diet.

Just as some compounds inhibit appetite, others are popularly supposed to increase it, such as pepper, mustard, cloves and paprika. As these are important in the preparation of various canned meats, a study was undertaken by Wangenstein at Minnesota to determine their effect on gastric secretion and mucosa. None of the above spices stimulate gastric secretion in dogs. Paprika is the only one of these spices which stimulates gastric secretion in man, although there is some evidence that pepper produces the same effect occasionally. Cloves and mustard depress gastric secretion to a slight extent. The only



spice stimulating appetite in man was paprika. Mustard, pepper and cloves seem to aid in the formation of ulcer in dogs when administered concurrently with histimine in beeswax. The concentration of spices was necessarily very high and produced no irritation if histimine was not also present. Hence, the spices do not seem to be contraindicated for use in rations.

One relationship of appetite to food intake observed on animals which has the most bearing on survival rations was the voluntary limiting by rats of their food to correspond to the available water. They also limited their water intake when the food was limited.

If individuals are to be subjected to adverse conditions, it is important to know whether or not they may be conditioned by a diet to withstand later stress. Samuels at the University of Utah has fed rats ad lib on equal caloric diets high in protein, carbohydrates or fat. These animals were later subjected to exercise and tests run on their metabolic processes. The blood sugar of fat-fed rats increased significantly over that of the animals on a high carbohydrate diet. The latter showed a drop in blood sugar. Evidently the fat-fed rats show a decrease in the ability of peripheral tissue to use sugar. When stress was applied to these animals in the form of fasting, the fat-fed rats survived longest and excreted less nitrogen. Carbohydrate-fed rats showed a terminal high nitrogen excretion. The blood sugar decreased in both cases. The rats surviving for the shortest period of time were those animals fed a high protein diet.

From the foregoing it would seem that a high carbohydrate content would be preferable for a survival ration. The earliest such ration was, in fact, pure carbohydrate. This was based on the fact that the oxidation of glucose in the body produces water, thus lowering the amount which must be taken in from outside sources. As protein metabolism products require water for excretion, this food source was kept at a minimum. Fat, although providing water and high energy value, is nauseating in concentrated amounts.

However, it began to be felt that as much protein as possible should be included in the ration in the interest of preventing marked loss of body protein. The big question was - how much protein could be incorporated for a water intake presumably averaging 800 cc. per day, and what source of protein would favor the attainment of nitrogen balance? This last factor involved the amino acid make-up of protein - and this was a field only recently explored, insofar as the familiar human foods were concerned. As a means of obtaining the needed information, several research projects were established. Within the past two years valuable information has been obtained, not only relating to these immediate problems, but also in the basic physiology of low-calorie, protein feeding. These will be discussed briefly.

Early in the program of investigation there emerged one finding that appears to be basic in feeding the small amounts of food characteristic of survival rations. Swanson, working at Iowa State College, found that egg protein apparently conserved body protein in protein-depleted animals, and causes an unexpectedly marked reduction in the

amount of urinary nitrogen excreted when egg is the sole source of protein. This work was then repeated and verified by Swanson, as well as by Allison and co-workers at Rutgers University, using rats and dogs as test animals.

It now appears that a survival ration can be devised which will minimize water requirements and provide approximately 1,000 calories per man per day. The details of the exact relationship of carbohydrate to fat to protein are approximately: carbohydrates 45%, fat 30%, and protein 25%.

The Quartermaster Food and Container Institute for the Armed Forces appreciates the opportunity to participate in this symposium on military physiology.





## QUARTERMASTER CORPS CLIMATIC RESEARCH LABORATORY

Harwood S. Belding, Director of Research

### Physiological Testing of Quartermaster Items

When the Climatic Research Laboratory was established late in 1942, those responsible for promoting it -- Colonel Georges Doriot, Lt. Colonel D. B. Dill, Captain Paul Siple and Lt. Colonel Bestor Robinson -- had sufficient vision to see that studies of man in relationship to his environment, and studies of the protective needs of man in stressful environments, could yield information which would improve the efficiency and comfort of the fighting man.

However, at that time I doubt whether they dared use this broad and generalized concept for selling the idea to their superiors. Rather, I think they showed that the Quartermaster General was badly in need of a quicker and better means of evaluating experimental clothing and personal equipment. I think they also showed that physiologists and biophysicists with a cold chamber at their disposal had been able to furnish data regarding relative comfort, tolerance time and fit of clothing and sleeping bags; furthermore, that these data were supported by objective data on skin and rectal temperatures and body heat production, which had been utilized for calculating the thermal insulation provided. I believe that this was the primary reason why the Climatic Research Laboratory was activated: to furnish information indicative of the relative worth of various items. This was a short cut to the field testing that is necessary before any item can be accepted for military use. For example, instead of providing many samples of each of the numerous different types of sleeping bags, different in material, shape, size and type of construction, for laborious and time-consuming field tests, the Quartermaster was able, by supplying the Climatic Research Laboratory with one or two samples of each type, to obtain data indicating which principles of construction were worthwhile. He then needed to supply large numbers of only two or three types for field testing. As most of you know, this turned out to be the principal type of activity of the Climatic Research Laboratory during the war years.

However, Colonel John Talbott, the war-time Commanding Officer and Director of the Laboratory, also was a man of vision because as he instrumented for requested tests of clothing for insulation, freedom of movement, ease of donning, moisture penetration and fit, and as he acquired equipment for tests of stoves, candles, ball-point pens, alarm clocks, goggles and tentage, Colonel Talbott was systematically setting up a laboratory well equipped for fundamental studies of man in his



environment. It may still be considered that this Laboratory "pays its way" by conducting tests, but since the war there has been greatly increased opportunity for performance of pointed basic research.

At the present time, The Climatic Research Laboratory is supported as the principal laboratory research facility of the Environmental Protection Section, and during the past year or so, we have not heard representatives of higher echelons of the Office of The Quartermaster General represent the Laboratory as primarily a testing organization.

### Mission of the Laboratory

Actually, the Environmental Protection Section, of which the Climatic Research Laboratory is a part, is as much concerned with geographic and climatic factors of the environment as it is with responses of man to those environments. However, you, as physiologists, will be more interested in the activities of the Human Biology Sub-Section. That Sub-Section is concerned with: a. actually measuring the effective stresses imposed on men by various combinations of climatic conditions; b. studying the short-term and long-term adaptations of men, particularly fighting men, to stressful environments; and c. studying principles of protection which may be effective for minimizing environmental stresses.

### Measurement of Effective Environmental Stresses

For some time biophysicists, climatologists and engineers have been studying the stresses imposed by various environmental factors. Much knowledge of the heat stresses resulting from various combinations of these factors is now available, as is information concerning conditions associated with thermal comfort.

Much less is known about factors of environmental cooling, especially for the heavily clothed man in extreme cold. We do know that in zero weather the presence of a wind makes it feel colder, and that incident sunlight makes it feel warmer. But does anyone know whether heat extraction from man is greater at zero with a 20-mile-an-hour wind blowing, or at minus 20 with a one-mile-per-hour wind blowing? Predictions can be made regarding climatic stresses by making assumptions that the body represents on the average a cylinder of a certain diameter, that the insulation on the cylinder has a certain value, and that a certain fraction of the surface of a real man is effective for heat losses by convection and radiation; but, except over a limited range of environmental conditions, these predictions have not been verified.

Two tools for determining effective cold stress on man are now being readied for use at the Climatic Research Laboratory. The first is the treated "Copper Man", developed during wartime for studies of thermal insulation provided by clothing. Combined thermal stresses of the environment can be determined by placing him out-of-doors,

dressings him in a standard clothing assembly, and determining what heat input is necessary to maintain his "skin" at some reasonable temperature, such as 92°F. Effects of varying single environmental factors can be determined by exposing the same copper man to controlled conditions in the Laboratory chambers. The second tool is a suit of electrically heated undergarments, to be covered by conventional garments. With this device, and suitable controls, it will be possible to determine the electrical energy that must be added to metabolic energy in order to maintain comfort under various environmental conditions. These metabolic energy productions plus electrical energy equals total environmental stress.

Of course, we have already attempted to make the same measurements on real men by following the rate of body cooling, and by determining how hard a man must work to maintain thermal equilibrium in various environments, clothing being fixed in amount. But these methods are laborious and the results are difficult to interpret. We have high hopes that our new tools will yield information on windchill and on effective gains and losses by radiation.

#### Determination of Adaptations of Man to Stresses

The second technical objective of our research, that of studying short- and long-term adaptations of men to stressful environments, requires collaboration of the physiologist, biochemist and biophysicist, as well as the psychologist. A small contribution to knowledge in this field is the study of "Long-Term Acclimatization to Heat"<sup>(1)</sup> by William Christensen, Director of this Laboratory during the early post-war period. In a study of physiological responses of 7 men who were exposed daily to high temperature and humidity over a seven-month period, it was found that pulse rates and rectal temperatures accompanying hard work did not vary significantly over the last 6 months, but that sweating decreased steadily from an average of 825 grams per hour at the end of the 1st month of exposure to an average of 640 grams at the end of the 7th month. The reason for this significant and continued decrease in sweating is still not clear.

The first line of defense against heat or cold, the vasomotor mechanism, has not been investigated extensively at this Laboratory for the past three years. The purpose of these studies has been a. to describe more fully the effects of environmental temperatures on peripheral blood flow; b. to determine the extent to which vasomotor responses are appropriate for meeting the needs of the individual; and c. to determine what mechanisms are involved in control of peripheral blood flow. Richard Day, while at the Laboratory, designed two air plethysmographs with double copper walls. These devices have proved useful for measuring blood flow and partitioning heat losses from the hands and feet at various controlled environmental temperatures. Blood flows as small as 0.15cc. per 100cc. of hand tissue per minute were recorded after exposure of the body to cold for several hours<sup>(2)</sup>. Under these conditions it is submitted that there must be considerable cooling of arterial blood before it enters the hand. This prediction has been



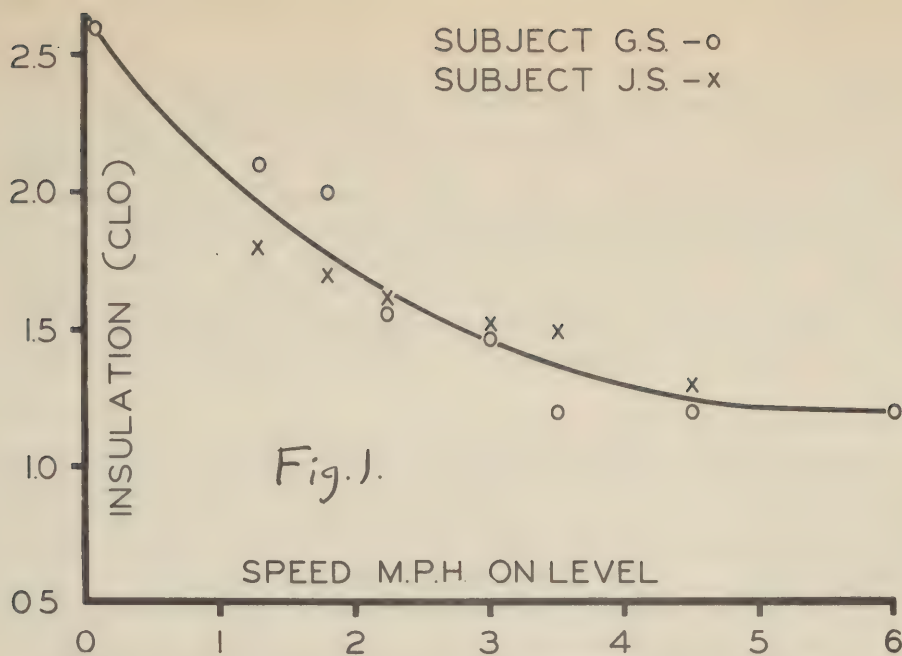
substantiated by H. C. Bazett and John Talbott with direct measurements of arterial blood temperature. The hand plethysmograph has also been used by Ferris, Forster, Pillion and Christensen<sup>(3)</sup> to investigate blood flow in both hands when heat is applied to one hand; effects of warming the feet on blood flow in the hands were also observed. It was found that, when a steady hand blood flow is reached at ambient temperatures which give rise to body cooling the blood flow to an unheated hand appears to be dependent on the overall need of the body for the conservation or dissipation of heat. In other words, if sufficient heat is applied to the legs or to the other hand to create an overall heat load on the body, vasodilatation of the unheated hand is induced.

It has been noted many times that in a cold environment ingestion of food results in a sensation of relative warmth which lasts for some time after eating. It was thought that this sensation might be related to increased peripheral blood flow, and that increase in peripheral blood flow might be related to the specific dynamic action of the foodstuffs. Accordingly, Martin Macht has recently conducted a study of "Effects of Ingestion of Certain Amino Acids on Peripheral Blood Flow". His results will be reported at a later session of these meetings.

#### Studies of Principles Involved in Providing Protection Against Stresses

We regard as of primary importance our responsibility for studying the principles which may be utilized to provide better protection against environmental stresses which may be faced by man. This Laboratory has relied, and must continue to rely, on work of other laboratories, military and civilian, for much of its information on environmental stresses and on adjustments of men to those stresses. The rate of our progress in the study and application of principles of protection depends in large measure on the rate of accumulation of the more basic information defining stresses and man's responses to them. Let me cite two examples of what I mean by this.

For the welfare of soldiers in the heat, we are interested in determining not only the relative heat loads imposed by various experimental and standard jungle and desert uniforms, but we are also interested in effects on heat load and subjective comfort of such specific fabric characteristics as density of weave, thickness, wetting, and color. In setting up our experiments to determine the heat load imposed by garment assemblies, we have relied heavily on results of work of the Medical Field Research Laboratory of the Aero-Medical Laboratory, and of E. F. Adolph's desert research group; they have given us the combinations of heat and humidity which will provide suitable stresses. Then we have depended on the studies of laboratory groups headed by D. B. Dill, Sid Robinson, R. E. Johnson, and on the publications of the Medical Field Research Laboratory to furnish us with information concerning the indices of heat stress. And finally we have used the data of all of these groups in interpreting our results.



**Fig. 2** RECOMMENDED ACTIVITY FOR COMFORT DURING A FIVE-HOUR EXPOSURE WIND 2 m.p.h. TURBULENT

AMBIENT TEMPERATURE IN TEST CHAMBER °F	WALKING / TOTAL PERIODS	HALF HOUR PERIODS									
		1	2	3	4	5	6	7	8	9	10
<b>LIGHT ASSEMBLY</b>											
+40	0/10						HOT				
+20	4/10						HOT				
0	5/5						MEAL				
-20	1/1						MEAL				
-33	1/1						MEAL				
<b>MEDIUM ASSEMBLY</b>											
+40	0/10						HOT				
+20	1/10						HOT				
0	2/10						MEAL				
-20	6/10						MEAL				
-33	8/10						MEAL				
<b>HEAVY ASSEMBLY</b>											
+40	0/10						HOT				
+20	0/10						HOT				
0	1/10						MEAL				
-20	2/10						MEAL				
-33	3/10						MEAL				



Oddly enough, we also use these same indices of heat stress for studies of performance of garment assemblies under cold weather conditions. It has long been observed in the field that a major problem is the accumulation of sweat in cold weather garments. Men sweat considerably when working hard in present Army cold weather clothing even in subzero weather, and studies at the Fatigue Laboratory have indicated that under these conditions men also exhibit the other established signs of heat stress: elevated rectal and skin temperatures, accelerated pulse rate, and sometimes even what we have termed the "hot room flush".

One of the principles recently studied under Quartermaster sponsorship relates to the fact that conventional cold weather clothing differs in the insulation which it provides when men are at rest and at work. A 3 clo cold weather uniform, so rated when a man is sitting quietly, is effectively a 1-1/2 clo uniform during walking<sup>(4)</sup>. (Figure 1). Movement of the layers of clothing resulting from body movements apparently stirs up the air trapped between layers with the result that convection losses are increased. Now, what we want is a cold weather uniform which will provide more insulation than at present when men are inactive and less than at present when they become active. Is it possible to develop a clothing assembly that will give up to 5 or 6 clo when men are inactive, and which will give decreased protection appropriate for maintaining comfort without sweating at different grades of activity?

Before we can intelligently exploit such principles as this for developing better clothing and personal equipment, we should have more precise information regarding the performance characteristics of items presently available. For example, clothing requirements for comfort are obviously dependent not only on the climatic conditions of exposure, but on the grade of activity being performed and on the length of exposure. The Quartermaster and the Surgeon General have shown cognizance of this by conducting studies of so-called Time-Energy relationships on field troops. Last spring we conducted what was in some ways a field experiment under controlled conditions in a Laboratory Cold Room to determine, with wind and temperature conditions constant, how much activity was necessary for men to maintain themselves in comfort for a five-hour period in three different assemblies of clothing; light, medium, and heavy. Some results of this study are indicated in figure 2.

### Field Studies

We are now operating a Field Laboratory in a higher latitude as an aid in solving some of our problems. Perhaps the majority of you who are assembled here today may be classed broadly as environmental physiologists. As such I am sure that you feel that no matter how well environments may be simulated in the Laboratory, it is still necessary to conduct field studies. Among the major objectives of our present venture into the field are:

a. To give Laboratory personnel a background of practical experience in cold weather living so that they may more intelligently plan and carry out laboratory research and testing.

b. To test the extent to which laboratory experiments performed on men and equipment are predictive of actual comfort of men and performance of items in the field.

c. To test the feasibility of making such objective measurements in the field as are made in the laboratory.

In conclusion, I would submit that present military problems represent a real challenge to the environmental physiologist. Not only must he acquire basic information on environmental stresses and adaptations of man, he must also discern the practical implications of his work. He is responsible for establishing the principles involved in providing protection and for contributing ideas exploiting those principles for improving the comfort and efficiency of the fighting man. And finally, his understanding of the principles makes him best qualified for studying and testing items recommended for military use.

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SCHOOL OF AVIATION MEDICINE

RANDOLPH FIELD, TEXAS

Colonel Don Flickinger, Director of Research  
Dr. Harry F. Adler

Effect of Altitude on Resistance and Immunity

Dept. of Bacteriology & Immunology

Phase I. The effect of altitude is being studied on the development and precipitation of allergic phenomena when the antigen is administered by mouth.

Hypoxia is known to increase tissue and cellular permeability and therefore a fed antigen may be absorbed before it can be digested thereby setting up an allergic state in a previously normal animal or precipitating symptoms in a sensitive one.

The method has been to use normal horse serum as the antigen in guinea pigs, subsequently exposing them to an altitude of 30,000 feet both in the sensitized and unsensitized state.

Our results thus far are as follows: (a) Horse serum and normal animal plus 30,000 feet for 5 hours leads to a constant development of an allergic state. Horse serum plus normal animal without flying only occasionally leads to sensitization. (b) Sensitive animal (by parental inoculation) plus fed antigen 4 weeks later plus immediate flight to 30,000 feet for 5 hours results in death of animal in most cases. Sensitized animal plus fed antigen and not flown do not die. Similarly a sensitive animal flown to 30,000 feet without antigen will not die.

We have interpreted these results as being due to a state of increased capillary permeability. However, the possibility exists that there may be inherent change in susceptibility to anaphylactic shock at altitude without regard to permeability and absorption. If the basis actually rests in the altered permeability state then the basic mechanism may be either (a) hypoxia (b) reduced pressure (c) intestinal distention. Further work is in progress attempting to separate out the factor of hypoxia by keeping animals under reduced pressure while breathing oxygen at ground level pressures.



Phase II. Effect of Altitude on Complement Level. Although the question is not finally resolved, it is thought that the ability of the organism to resist infectious disease is reflected in the complement level. It is also known that complement level is at least partially dependent upon liver function which may well be impaired under conditions of hypoxia.

The preliminary results thus far have shown a statistically significant drop in complement level in a group of rabbits flown continuously at 15,000 - 20,000 feet. Animals brought back to sea level are now showing a gradual return to baseline values.

Phase III. Effect of Altitude on Ability to Produce Antibodies. This work has just been started with rabbits using Eberthella typhosa as the immunizing stimulant. Thus far the results have shown no difference between those animals immunized on the ground and those at altitude.

Phase IV. Effect of Altitude upon the Severity of an Induced Bacterial Respiratory Disease and an anaerobic Infection. Work has just been begun on this project and no results are as yet available. At present time our techniques for standard inoculation are being perfected.

#### A Study of the Role of Respiratory Enzymes in Anoxia

##### Department of Biochemistry

The activities of the Biochemistry Department have centered on the role of respiratory enzymes in anoxia. The goal of such investigations is to determine whether any component of these enzymatic systems can be used to increase resistance to anoxia. The first substance examined was cytochrome C, in view of the reports of Proger and Dekanaes that it's injection increased the body's resistance to anoxia. We have been unable to confirm this work. Cytochrome C added to tissue slices caused no increase in oxygen uptake. Furthermore, our data suggest strongly that injected cytochrome does not penetrate the cell. This is a crucial point, for if cytochrome does not enter the cell, it is extremely difficult to visualize how it can be of benefit. To gain further information on this point, experiments are now in progress in which the cytochrome content of one kidney is measured before cytochrome injection and that in the remaining kidney after injection and perfusion. Preliminary results show little if any cellular penetration. Studies with cytochrome containing radioactive iron will be started shortly to gain further information on this point.

It was next decided to study the effect of succinate utilization in anoxia. This substance was chosen since it has a small molecular weight, is freely diffusible into most tissues and has a vital role in cellular respiration. It was found to increase markedly the oxygen uptake of slices of kidney, liver and heart but not of brain, respiring in air and in 8% oxygen. We are now investigating whether this increased oxygen uptake can be utilized as energy by the body.

This necessitates a study of the high energy phosphate compounds in the tissue in anoxia with and without prior injection of succinate. Our results to date are inconclusive.

A technique is being developed in conjunction with the Department of Neurology to obtain a satisfactory, objective measure of anoxia. The method consists essentially of recording the electroencephalograms of unanesthetized rabbits during periods of nitrogen breathing. The time required to produce typical changes in the electroencephalogram is determined for each rabbit. After a recovery period, the rabbit receives the substance to be tested, nitrogen is again given and the EEG response again measured. Simultaneous determinations or recordings are made of arterial and sinus oxygen content, glucose, hemoglobin, body temperature, electrocardiograph, alveolar carbon dioxide and respiration. Cytochrome injection had no effect; succinate, pyruvate and fumarate had slight beneficial action. These conclusions are tentative since the technique has not been adequately standardized as yet. A large series of enzymes, substrates and poisons will be studied by this procedure.

#### Effects of Reduced Atmospheric Pressure on Excretory Function of the Kidney

##### Department of Experimental Medicine

At the present time there is a paucity of data in the medical literature on the effect of reduced atmospheric pressure on the excretory function of the kidney. Alving and coworkers (CAM report No. 135, June 16, 1943) studied the effects on renal function of exposure of young men to simulated altitudes of 10,000 to 18,000 feet for 4-6 hours daily, 6 days per week over periods of 4-6 weeks. Renal function was studied at various times, the earliest tests being on the 13th day of exposure and the latest ones on the 41st day of exposure. They found no changes in renal blood flow, glomerular filtration rate, nor filtration fraction but did find an increased maximum tubular excretory ability for diodrast in every case studied.

There are no reports in the literature of the effects of acute exposure to altitude on these functions. The Department of Medicine of the School of Aviation Medicine has been and is continuing to investigate this phase of renal physiology. By the non-surgical techniques developed by Homer Smith and his collaborators at New York University, the effects of acute exposure to simulated altitudes on glomerular filtration rate, renal blood flow, filtration fraction, maximum tubular excretory ability, and maximum tubular reabsorptive capacity are being studied.

Results obtained in these studies indicate that the increased



maximum tubular excretory ability observed by Alving in the studies previously mentioned also occurs upon acute exposure to severe altitude hypoxia. In only one case was such an increase observed immediately upon exposure to 18,000 feet but at 24,000 feet 2 of 5 animals showed elevation of maximum tubular excretory ability to values approximately three times their ground level norms.

It was found that total renal blood flow varied with the altitude to which the animal was exposed in such fashion that it increased progressively with increasing altitude to a maximum value and then decreased at higher altitudes, so that at 24,000 feet in only one case was the renal blood flow still as great as the ground level norm.

Glomerular filtration rate exhibits only small variations upon acute exposure to altitude. Since no pattern of variation is evident in this function for all practical purposes glomerular filtration rate may be considered to be unaffected by exposure to altitude. As a corollary of the two conclusions that total renal blood flow is affected by altitude, and that glomerular filtration rate is not affected by altitude it may be surmised that the filtration fraction is altered by altitude since filtration fraction is by definition the ratio of glomerular filtration rate to total renal blood flow. It was found in these studies that in every determination at altitude the filtration fraction varied significantly from the ground level norm.

In contrast to the observation of increased maximum tubular excretory ability it has been observed that acute exposure to altitude causes consistently a decrease in the maximum tubular reabsorptive capacity. When these two functions are simultaneously measured there results at ground level a marked depression of maximum tubular excretory ability and either an unchanged or a slightly depressed maximum tubular reabsorptive capacity as compared to the values obtained when these functions are measured individually. Under these conditions of simultaneous determination exposure to altitude does not cause the marked elevations of maximum tubular excretory ability noted when that function is measured alone but does cause a depression of the maximum tubular reabsorptive capacity below the corresponding value at ground level. This depression, however, is not as pronounced as is the corresponding depression when the maximum tubular reabsorptive capacity is measured alone.

Adequate evaluation of the significance of these observations to aviation medicine will be possible only after more thorough and exhaustive investigation. However, from the evidence thus far obtained it would appear that exposure of an individual to moderate altitude hypoxia enhances his kidney function rather than affecting it adversely as might be expected. On the other hand exposure to extreme altitude, because of the result and decrease in total renal blood flow, must in all probability be considered detrimental to proper renal function.

## STUDIES ON ASPHYXIA I.

This first study (1) was to obtain information on the acid-base balance of the blood during obstructive asphyxia and asphyxia produced by administration of 100% nitrogen.

Dogs were used throughout the experiments. They were anesthetized with nembutal at a 'physiologically standardized' dosage in order to avoid undue depression of the medullary centers. The control values for arterial pH and  $pCO_2$  were within the normal range and indicate that there was little or no respiratory depression in these animals. The trachea was cannulated and attached to a calibrated McKesson anesthesia-machine which provided any desired mixture of oxygen and nitrogen. By means of a three-way valve, the animal could be disconnected from the anesthesia apparatus and attached instead to a positive pressure pump for artificial respiration. The length of stroke and rate of this pump could be varied to give any desired value for tidal air and minute volume. Respiratory minute-volume was recorded by passing the expired air through a low resistance gas meter provided with contact points to record electrically on a smoked drum. Respiratory rate was recorded by means of a pneumograph and tambour. Blood pressure was recorded from the femoral artery. Ten cc. samples of circulating blood were obtained from a 'T' cannula in the carotid artery, the animals having been previously heparinized in order to prevent clotting. The blood was run under oil into a special centrifuge tube containing fluoride and was centrifuged immediately in the completely filled stoppered tube as described by Peters and Van Slyke. The plasma was then stored over mercury until the analyses were performed. All analyses were completed within 4 hours. The last few drops of each blood sample were run into a separate vessel and used for hemoglobin determinations.

Total plasma  $CO_2$  content was determined by the manometric method of Van Slyke. Plasma pH was measured at  $37.5^\circ C$  by means of a high resistance, syringe type glass electrode in conjunction with a Leeds and Northrup type  $K_2$  potentiometer and a #7673 thermionic amplifier. This method has a maximum error of  $\pm 0.01$  pH unit. Hemoglobin was determined colorimetrically in an Evelyn photoelectric colorimeter.

From the measured values of plasma  $CO_2$  content and pH, the  $pCO_2$  and  $BHCC_3$  content were calculated according to the Henderson-Hasselbach equation:

$$\begin{array}{rcl}
 & (CO_2) & \\
 & \hline
 pCO_2 = & 0.0672 \left( \frac{7.9 \times 10^{-7}}{(H^+)} + 1 \right) & \dots 1 \\
 & & \text{and} \\
 BHCC_3 = & (CO_2) - 0.0672 pCO_2 & \dots 2
 \end{array}$$



In addition, plasma bicarbonate capacities for fully oxygenated blood at a standard pH of 7.40 were calculated according to the following equation:

$$(\text{BHC}\text{O}_3)_{7.40} = (\text{BHC}\text{O}_3)_{\text{pH}} + (\text{pH} - 7.40) (16.3 + 2.3 \text{ Hb}_B) - 0.36 (\text{Hb}_B - \text{O}_2(B)) \dots 3$$

where  $(\text{BHC}\text{O}_3)_{7.40}$  is the plasma bicarbonate capacity at  $\text{pH}=7.40$ ,  $(\text{BHC}\text{O}_3)_{\text{pH}}$  is the plasma bicarbonate content at the observed pH,  $\text{Hb}_B$  is the oxygen capacity in volumes % and  $\text{O}_2(B)$  is the oxygen content in volumes %. This equation corrects the observed bicarbonate content for changes in pH, hemoglobin content, and oxygen saturation and is important in the proper interpretation of acid-base changes during asphyxia. The constant, 16.3 in equation 3, which represents the value of  $\Delta \text{BHC}\text{O}_3$  for plasma alone, is based upon human plasma containing 70 grams of  $\Delta \text{pH}$  protein per liter with a normal albumin-globulin ratio of 1.6. Its exact value will vary with the total protein content and the albumin-globulin ratio. The value 16.3 should apply approximately to normal dog plasma in which the total protein content and the albumin-globulin ratio do not differ greatly from those of human plasma. It is to be remembered, however, that calculations based on equation 3 will give approximate values only in the absence of exact data for the values of the constants in each case.

### Summary of Findings

1. Acid-base changes in the arterial blood of dogs during fatal asphyxia and during successful resuscitation were studied. Asphyxia was produced by tracheal obstruction or by nitrogen inhalation.

2. During fatal obstructive asphyxia, there was an early respiratory acidosis followed by an uncompensated metabolic acidosis. By the time circulation failed, arterial  $\text{pCO}_2$  reached an average value of 64.9 mm Hg, and the pH fell to 7.20.

3. In fatal nitrogen asphyxia, the early respiratory alkalosis was overcome by a late respiratory and metabolic acidosis so that both arterial  $\text{pCO}_2$  and  $(\text{H}^+)$  were slightly above normal when circulation failed.

4. During artificial respiration with air at the animal's control rate and tidal volume, arterial  $\text{pCO}_2$  and  $(\text{H}^+)$  continued to rise in most cases. In all cases, both the  $\text{pCO}_2$  and  $(\text{H}^+)$  remained above normal until spontaneous respiration was resumed. On restoration of spontaneous breathing, hyperventilation occurred.

5. These results indicate that there is no deficiency in the chemical stimuli to the respiratory center during the entire course of obstructive asphyxia or in the later stages of nitrogen asphyxia. They further indicate that artificial respiration with air at a normal minute volume does not produce a deficiency in the levels of these agents in the arterial blood. Thus it would appear to be

unnecessary or even harmful to include  $\text{CO}_2$  in the resuscitative mixture under these conditions.

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#### STUDIES ON ASPHYXIA II.

Our base level studies on asphyxia and the results of a number of previous investigations indicate that the ability of carbon dioxide to stimulate breathing may be greatly decreased and occasionally even reversed under conditions in which the respiratory center is depressed by narcotic drugs or anoxia. In view of the possible implications in regard to resuscitative procedures the following study was undertaken to extend the observations of Schmidt and to obtain additional data on the respiratory and circulatory responses to  $\text{CO}_2$  inhalation during severe acute anoxia. Also, in most studies,  $\text{CO}_2$  was given together with high concentrations of oxygen so that it is possible that some of the effects observed may have been partially due to oxygen inhibition.

Dogs lightly anesthetized with nembutal were used. Respiratory rate was recorded by means of a pneumograph and tambour. A valved tracheal cannula allowed the expired air to be passed through a low resistance gas meter to provide a record of respiratory minute volume. Arterial blood pressure was recorded from the femoral artery with a mercury manometer.

Gas mixtures were administered through a standard AAF A-12 'Airco' demand regulator. Four different mixtures were used: No. 1. 20%  $\text{O}_2$ , 5%  $\text{CO}_2$ , 75%  $\text{N}_2$ ; No. 2. 4%  $\text{O}_2$ , 96%  $\text{N}_2$ ; No. 3. 4%  $\text{O}_2$ , 5%  $\text{CO}_2$ , 91%  $\text{N}_2$ ; No. 4. 4%  $\text{O}_2$ , 15%  $\text{CO}_2$ , 81%  $\text{N}_2$ . The experimental procedure was carried out as follows: First, control records were obtained with the animal breathing air. The dog was then placed on mixture No. 1 for 2-3 minutes to determine the ability of  $\text{CO}_2$  to stimulate breathing in the animal before the induction of anoxia. If a significant stimulation was not obtained under these conditions, the animal was not used. After thus establishing the response to 5%  $\text{CO}_2$  in the non-anoxic state, the dog was placed on mixture No. 2 (4%  $\text{O}_2$ , 96%  $\text{N}_2$ ) and subsequently did not



inhale more than 4% oxygen unless resuscitation became necessary. The response to the inhalation of mixture No. 3 (4% O<sub>2</sub>, 5% CO<sub>2</sub>, 91% N<sub>2</sub>) was tested at intervals of 15 minutes during the course of the anoxia. Mixture No. 4 (4% O<sub>2</sub>, 15% CO<sub>2</sub>, 81% N<sub>2</sub>) was tested at irregular intervals during the course of the experiment.

#### Summary of Findings

1. The respiratory and circulatory responses to carbon dioxide (5%, 6%, 15%) were tested in 14 dogs during the course of acute severe anoxia produced by the inhalation of 4% oxygen.
2. Under these conditions, the stimulatory effects of CO<sub>2</sub> progressively decreased, soon disappeared, and were finally reversed.
3. Carbon dioxide inhalation during anoxia produced respiratory depression in eleven tests on five animals under conditions such that it could not have been due to anoxic collapse or to 'oxygen inhibition'.
4. Fifteen per cent CO<sub>2</sub> produced more frequent and more profound respiratory and circulatory depression than did 5% CO<sub>2</sub> when administered at the same stage of anoxia.
5. Five per cent CO<sub>2</sub>, when given at the beginning of anoxic collapse, had no demonstrable effect on blood pressure or respiration.

#### References

Ivy, John H.; Grodine, Fred S.; Adler, Harry F., and Snapp, Forrest E.: Studies on Asphyxia. II. Effects of Carbon Dioxide Inhalation on an Anoxic Animal. Project No. 474, Report No. 2, AU School of Aviation Medicine, Randolph Field, Texas, 17 July 1947.

#### STUDIES ON ASPHYXIA III.

We have initiated this project on dogs in an attempt to evaluate several agents which are known stimulants to respiration or are known to prolong survival time in asphyxia and hence may possibly be used in resuscitation in addition to artificial respiration.

We have carried out the following experimental work in an attempt to evaluate certain known stimulants in regard to their value in resuscitation. Our procedure has been to use dogs lightly anesthetized with nembutal. Continuous records were made of arterial blood pressure, respiratory rate and excursion and respiratory minute volume. Blood samples were taken from the carotid artery for determination of the oxygen content, hemoglobin and blood sugar level. After control determinations were made the animal was exposed to an atmosphere of 100% nitrogen until cessation of respiratory movements. Blood samples were again drawn and a stimulant given intravenously. If the animal

failed to revive in a short time, it was given artificial respiration using 100% oxygen. Usually the same animal was used several times.

The drugs tested were coramine, metrazol, alpha lobeline, and amphetamine sulfate. We attempted to use a dosage in each case which was high enough to produce stimulatory effects if possible, but not high enough to produce toxic effects. The doses were chosen on the basis of our preliminary tests on dogs under nembutal anesthesia, and on the experiments described in the literature by other workers.

### Results and Conclusions

One hundred and nine periods of anoxia were produced in 38 dogs. Twenty of the dogs in 56 of these tests never showed a single instance of spontaneous revival whether they received medication or not. The other 18 dogs revived without the use of artificial respiration in one or more periods of anoxia. In only 9 of these dogs, 2 receiving metrazol and 7 receiving amphetamine sulfate, did the revival appear related to the drug administration. Further study of these records and more experiments are necessary before attempting to evaluate these findings.

What about the possibility of the injection of one of these drugs causing actual damage to the animal? Several dogs receiving the higher doses of the drugs used died in spite of the fact that artificial respiration was begun early in some cases even spontaneous respiratory movements were made. Coramine apparently contributed to death in two dogs with doses of 125 and 156 mg. per kilo; metrazol in two dogs with doses of 33.9 and 45.8 mg. per kilo; and alpha-lobeline in one dog with a dose of 0.86 mg. per kilo. One must consider, of course, that other factors such as repeated periods of anoxia and previous injections of drugs may have had something to do with these deaths.

The time between exposure of a dog to 100% nitrogen and the onset of apnea was used as a measure of the resistance of the animal's respiratory center to anoxia. Observation of blood pressure fall is also used as an indication for beginning artificial respiration. Nine of the dogs were given 10% glucose solution intravenously for a period of at least an hour before exposure to anoxia. This treatment was found to have no significant effect on this period of survival. The blood sugar levels during the periods of anoxic apnea showed no consistent change over the levels during control periods in either the dogs receiving glucose or those not receiving it. There were some elevations of sugar level and some depressions while many dogs showed no change.

In summary our results indicate that, in the dosages used, coramine and alpha lobeline were not effective in stimulating dogs made apneic by anoxia. Spontaneous revival apparently related to the



administration of the drug was noted in 2 dogs receiving metrazol and 4 dogs receiving amphetamine sulfate. Drug administration has not been consistent in causing stimulation of the respiratory center in dogs depressed by anoxia. The ease with which dogs were revived with artificial respiration and 100% oxygen given early enough before circulatory collapse shows that one most important factor in resuscitation is oxygenation. The respiratory center depressed to the point of apnea by oxygen lack does not respond readily to drug stimulation, just as it does not respond to the physiological stimulants already present. Evidence was obtained indicating toxicity of the drugs in the higher doses used.

Blood sugar levels during periods of anoxic apnea showed no consistent change over levels during control periods. Administration of intravenous glucose had no apparent effect on the resistance of the animal's respiratory centers to anoxia.

Equipment for following the blood oxygenation continuously during experiments is being developed. A small photoelectric cell is activated by light transmitted through the carotid or femoral artery of the dog and after amplification the current is recorded by an ink-writing galvanometer. The apparatus is being checked against Van Slyke oxygen determinations and studied for its applicability in respiratory research.

With this device standardized by the chemical determinations it has been possible to follow the moment to moment changes in oxygenation of the blood. Our blood studies have been extended beyond those previously mentioned to include also the determination of oxygen capacity, the carbon dioxide content of whole blood (arterial) and plasma, and the plasma hydrogen ion concentration. In addition alveolar samples are analyzed for CO<sub>2</sub> and O<sub>2</sub> concentration. These various measurements are made on blood and alveolar air samples collected simultaneously under different conditions of respiration. It is hoped that data collected by these and other contemplated methods may be of value for several purposes: (1) to show some of the changes which occur in hypoxia and anoxia, (2) to study the mechanisms of recovery from asphyxia, (3) to study the physiology of respiration, for example, the relationships between the pH of blood and the concentrations of oxygen and carbon dioxide in blood and alveolar air.

More specific problems are found under the subject of resuscitation. In revival of the apneic asphyxiated animal the objective is the reinstituting of respiratory movements in the animal in an atmosphere of adequate oxygen concentration. In some animals respiratory movements begin spontaneously while in others use of artificial respiration is necessary for varying periods of time before spontaneous movements begin. Study of these recoveries may be useful in developing methods of restoration of spontaneous respiratory movements early.

## EFFECT OF EXPLOSIVE DECOMPRESSION ON VITAMIN C DEFICIENT GUINEA PIGS

Because of its obvious importance in Aviation Medicine, it is desirable to investigate any factor which might exacerbate the theoretical or actual tissue damage due to explosive decompression.

Capillary fragility due to vitamin C deficiency may be one factor in tissue damage due to explosive decompression. It is justifiable to investigate this factor because manifestations of vitamin C deficiency with bleeding of the gingiva has been noted in Altitude Chamber Personnel repeatedly exposed to anoxia. Conceivably, flying personnel could likewise be susceptible to vitamin C deficiency and hence might suffer more severe damage than the normal individual if explosively decompressed.

A preliminary trial on 10 guinea pigs has been completed. Five guinea pigs were given a normal diet and 5 guinea pigs were placed on a vitamin C deficient diet for 25 days. At this time there was a definite increase in the number of petechial hemorrhages in the skin of the vitamin C deficient guinea pigs when a decrease in pressure of 250 mm Hg for 1 minute was applied. The guinea pigs were then explosively decompressed from ground level to a pressure altitude of 67,000 feet, and autopsied during the next three days. The time of decompression has not yet been calculated. No guinea pigs died as a result of the explosive decompression. When sacrificed, hemorrhages were found in only the vitamin C deficient guinea pigs. These hemorrhages occurred in the lungs, viscera, abdominal wall and the thoracic wall. On the basis of these results the materials are being assembled for further experiments.

### EXTENT OF PATHOLOGICAL DAMAGE TO ANIMALS IN A COLD ENVIRONMENT WHO ARE EXPLOSIVELY DECOMPRESSED

Planes which fly at very high altitudes will be pressurized with air to a value as close as possible to that existing at sea level (760 mm Hg). Under these conditions, the plane, for example, may be flying at 40,000 feet where the external environment of the plane will be about 140.7 mm Hg, but the plane's cabin and hence its occupants will be kept pressurized at an altitude of 8,000 feet (564.4 mm) by special compressors. Such pressurization is advantageous because it prevents symptoms of oxygen lack and decompression sickness.

This arrangement immediately exposes the plane's occupants to the possibility of "explosive decompression", which is the name given to what occurs when an aircraft with a pressurized cabin is damaged



in flight so that the cabin air suddenly drains out until it equalizes with the low atmospheric pressure outside. Under the experimental conditions used such very rapid decreases in pressure have not so far been particularly harmful to human subjects.

All experiments carried out to date, however, have been done at normal room temperatures, a circumstance which probably operates in favor of the subject being decompressed. Under actual flying conditions the passenger would not only experience a large pressure change, but at the same time he would be exposed to an extremely low temperature. There appears to be little reason to expect that the changed physical characteristics of the air caused by the frigid temperature would materially alter the rate or violence of the decompression. On the other hand, it seems entirely possible that in the event of lack or failure of cabin heating, flying personnel might become so cold that they would not be able to tolerate explosive decompression as well as in warm air because of their changed physiological state. Factors introduced by the cold might include such considerations as a more tense chest and abdominal wall, a partially closed glottis, shifting of the blood volume to the interior of the body, and so on.

It is, therefore, proposed that an investigation into the relative effects of explosive decompression on animals in warm and cold air be carried on to determine whether the results of decompression in the cold are more severe. In doing this work it would seem desirable to subject the animals for varying periods of time to temperatures corresponding to those found in the interior of unheated planes flying at 25,000 to 40,000 feet in the frigid zones, just before the explosive decompression tests. Pathological results and subsequent clinical behavior would be the main criteria of severity of the effects.

In connection with these experiments on explosive decompression a small but versatile "parasite chamber" of about 35-40 cubic feet capacity is being planned which will allow not only direct observation of experimental subjects, but also objective methods of recording physiological changes such as by photography, electrocardiograms, electro-encephalograms, x-ray, etc.

In addition devices are being developed for:

(1) Continuous registration of respiratory volume under varying pressures by photo-electric means and a stepping relay.

(2) Immediate determination of water vapor pressure in samples of alveolar air.

(3) Modification of the oximeter by insertion of rotating filters so that oxygen content and saturation can be obtained from the same area. This modified system will also allow one photo-cell and one galvanometer to be used.

## THE EFFECT OF PRESSURE BREATHING ON INTRACRANIAL PRESSURE

This problem assumes practical importance because it is possible that pressure breathing devices will be used on patients whose respiration is embarrassed, and that some of these patients might have head injuries. It is known that positive pressure applied to the lungs causes some degree of obstruction to cardiac venous return. This obstruction could be reflected back to the intracranial pressure and theoretically might cause exacerbation of an elevated intracranial pressure which already existed or was developing. In view of these concepts it would be desirable to know if the use of pressure breathing was contraindicated in head injury cases. At the same time the effect of the administration of 100% O<sub>2</sub> at ambient pressures and under increased pressures was studied because of a report to the effect that the arterial oxygen saturations were decreased in head injuries.

The animals were anesthetized with nembutal and a cannula was fitted into the skull. By raising a reservoir system filled with saline connected to a cannula and a manometer, the intracranial pressure could be artificially increased and measured. In all the experiments the blood pressures, intracranial pressures, and respirations were recorded. Blood samples were taken from the carotid artery. In most cases pressure breathing was administered with a General Electric Pneumolator at 18 cm of water pressure and with a Burns Resuscitator at 20 cm of water pressure. In a few cases the General Electric Pneumolator was used with pressures varying between 30 and 60 cm of water.

### Summary of Findings

1. The results on 25 dogs indicate that intermittent pressure breathing at 20 cm of water pressure in the presence of an elevated intracranial pressure will not exaggerate the mean intracranial pressure but may increase the fluctuations with respiration. These fluctuations with respiration were as high as 35 mm of saline, but 93.2% were 10 mm or below and 77.7% were 5 mm of saline or below. When the pressure in the pressure breather was increased to 30 cm of water and above there was a depression in the arterial blood pressure which did not occur when the pressure breather was set at 20 cm of water pressure.

2. A statistical analysis of the effect of various intracranial pressures and inhalants such as air, 100% O<sub>2</sub> and 100% O<sub>2</sub> under 20 cm of water on the arterial oxygen saturation and contents was done. This analysis reveals that when the animal was breathing air, both the arterial oxygen saturation and content were significantly decreased if the intracranial pressure is elevated so as to approach the diastolic blood pressure.



3. This reduction in arterial oxygen saturation and content occurs in conjunction with a significant reduction in the respiratory minute volume.

4. This reduction in arterial oxygen saturation and content can be remedied by the use of 100% O<sub>2</sub> and 100% O<sub>2</sub> under 20 cm of water pressure.

5. While there is no significant difference between the effect of 100% O<sub>2</sub> and 100% O<sub>2</sub> under 20 cm of water pressure on the arterial oxygen saturation and content, the resuscitator effect of the pressure breather has the additional beneficial effect of maintaining respiratory ventilation in the presence of apnea.

NAVAL MEDICAL RESEARCH INSTITUTE, NATIONAL

NAVAL MEDICAL CENTER, BETHESDA, MARYLAND

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Director of Research

The Naval Medical Research Institute, commissioned October 27, 1942 is engaged in basic and applied research directed to the betterment of efficiency and health of naval personnel and to problems inherent in the treatment of the sick and injured.

The organization (fig. 1) provides for three senior administrative officers, the Medical Officer in Command, the Research Executive, who coordinates the professional activities of the various investigators and the Executive Officer who supervises the various administrative units or facilities. The present staff consists of 63 investigators and about 75 technicians.

Research problems are formulated as projects and each project is placed in charge of a principal investigator. He thus becomes the key man for the progress of research and all available professional and administrative aid is directed to provide him with the freedom and stimulation requisite for the pursuit of scientific endeavor.

Certain studies may be selected which are representative of the type of physiological investigation carried out during the past 5 years.

Studies of Physical Fitness. The contribution of this Institute in the rather elusive quest for indices of fitness was the demonstration that a correlation does not exist between pulse rate response after moderate exercise and endurance performance. The validation of submaximal exertion tests against treadmill endurance performance has no physiological basis.

The value of pulse rate response of groups of individuals following moderate exercise was further demonstrated. Of interest also is the progressive decrease with age of endurance performance of homogeneous groups of men undergoing the same type of military training.

Studies of the Effects of High and Low Temperatures. The extensive temperature studies of Spealman, Pace and others led to the better protection of men immersed in cold water, and to the installation of air cooling equipment in the living spaces of ships operating in tropical waters. Spealman showed that minimal blood flow occurred when a hand or foot was immersed in moderately cold water (15° to 20° C) and



that blood flow was much greater at lower temperatures. Hence vaso constriction was not a factor in the production of 'immersion foot'.

In the hot temperature studies the conditions for the development of heat rash were found to be associated with dry bulb temperatures above 85°F and relative humidities of the order of 70 to 80%. The limits could therefore be set for maximal 'effective temperatures' that would not cause sweating of individuals at rest.

Of particular interest was the demonstration that mean basal oral temperature showed about the same degree of statistical stability as does rectal temperature. Average basal oral temperatures should therefore prove to be extremely useful in future shipboard studies. Further it was found that a decrease in basal oral temperature of only 1.0°F and even 0.5°F was associated with a relatively dry skin and heat rash associated with the higher temperature level.

The hot temperature studies demonstrated the remarkable consistency of results which can be obtained with properly conditioned men undergoing an unvarying routine. The men become in effect 'comfortimeters' and their physiological responses are of greater value than the recordings of instruments or the complicated formulation of environmental conditions in mathematical terms.

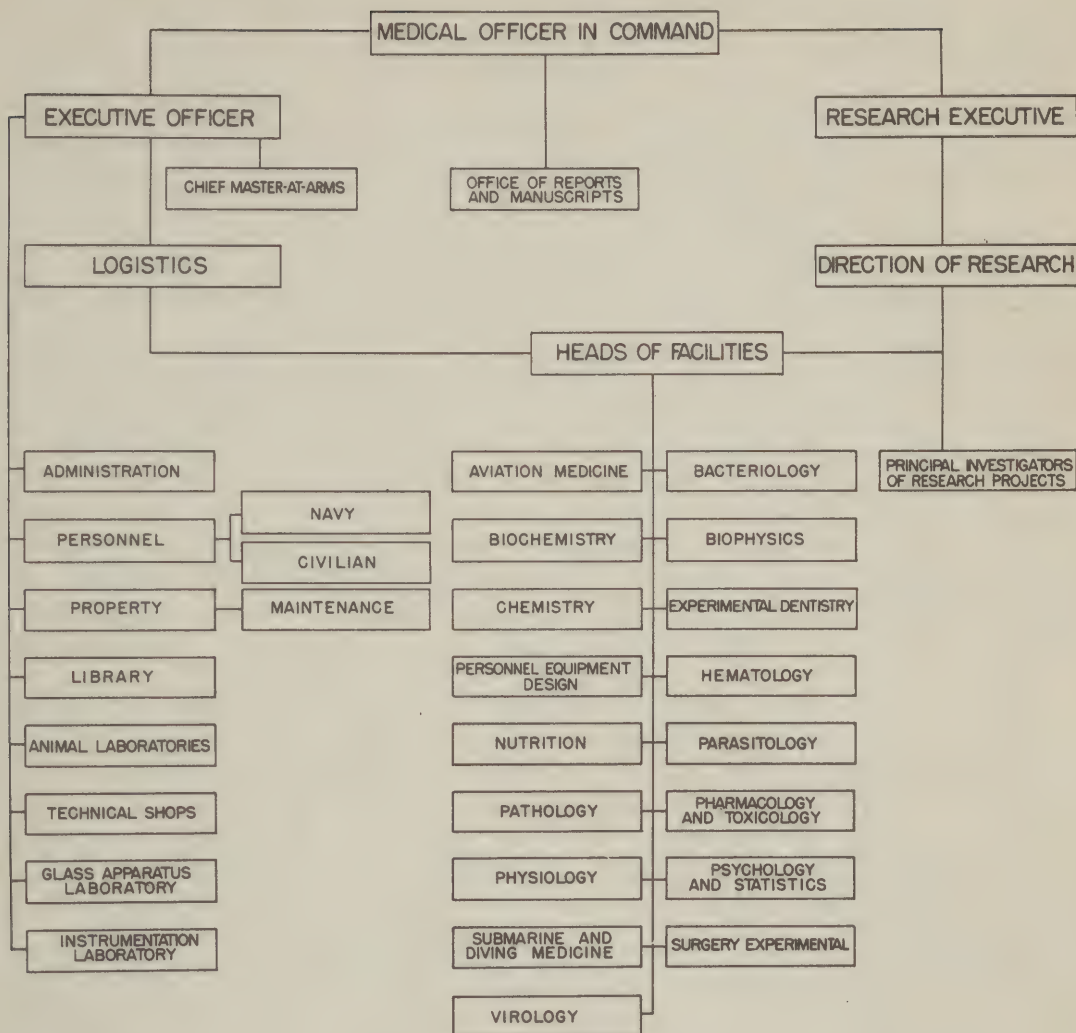
Minimal Salt and Water Requirement of Fasting Men. The problem of prolonging survival after ship wreck early in the last war led to a consideration of chemical methods of desalinating sea water. The first approach to the problem was to determine how much salt could be left in drinking water. Two groups of men placed on a diet largely sucrose were restricted to an intake of about 400 calories per day and allowed 500 ml and 1,000 ml of water. One half of each group had its water slightly salted (0.3 to 0.4 gms of sodium chloride per 100 ml), the other half drank distilled water. The men drinking distilled water excreted between 3 and 4 grams of sodium chloride daily. The group drinking 500 ml of slightly salted water conserved 230 ml of water per day more than the group that drank 1,000 ml of distilled water. By allowing 0.3 to 0.4% salt to remain in drinking water the fasting body requirement for salt was satisfied and a considerable reduction was effected in the daily water requirement.

Body Composition - Fat, Water and Nitrogen Content. The concept that the body consists of a lean mass of constant composition, and that accumulated fat is the major component responsible for alterations in specific gravity was supported by the findings of Rathbun and Pace. Using adult guinea pigs these investigators obtained the same range of values, 1.027 to 1.097 as was found for man. Aliquot fat free samples of pulverized body tissues including bone yielded a value of 1.098. Furthermore the inverse relationship postulated for man as existing between specific gravity and fat content was established for the guinea pig.

The concept that the lean body mass is of uniform composition it is further supported by the findings of Pace and Rathbun that the

Fig. 1

# NAVAL MEDICAL RESEARCH INSTITUTE ORGANIZATION CHART





percentages by weight of water and of combined nitrogen in aliquot samples of pulverized fat-free tissue show remarkably little variation.

Use of Radioactive Hydrogen for Measurement in vivo of Total Body Water. The problem of in vivo determination of total body water in man was approached using the radioactive isotope of hydrogen (tritium) in the form of water. The tritium was obtained by cyclotron bombardment of beryllium and was then converted to water. A method was developed for the determination of the activity of this water. This involved modification of the Geiger-Muller counter tube so as to permit introduction into the tube of measured amounts of radioactive water vapor, alcohol and argon. It was possible to evacuate and rinse the tube before each determination. Water of known activity was injected into two rabbits and one man and the plasma activity determined after a period of equilibration (close to one hour in the man). The body water of the human subject determined in this way agreed to within less than 1% with the value calculated from specific gravity assuming body water to constitute 73.2% of the lean body mass. The values obtained for the two rabbits agreed to within less than 5% with the water content determined by desiccation and weighing.

Effects of Decompression from High Pressure Atmospheres-- Studies of Gersh. As a result of too rapid decompression from high pressure atmospheres it was observed that bubbles were present intravascularly and in fat tissue or tissues as the adrenal gland that are rich in fat.

Employing the method of specific gravity Gersh demonstrated that a good approximation of the degree of bubble formation could be determined in the body as a whole and in tissues by measuring the changes in body density before and after decompression.

Further information was obtained regarding the etiology of chokes in the histologic demonstration that gas bubbles cause marked stretching of the walls of medium sized and small pulmonary arteries. It will be recalled that substernal distress during deep inhalation is a diagnostic symptom of incipient chokes. In the anesthetized dog chokes is characterized by a tachypneic type of breathing. The question arises does deep inhalation serve to distend further the gas filled pulmonary vessels? And would not measurements of impulses from periarterial sensory fibers demonstrate an augmented output during the inspiratory phase of the respiratory cycle.

Measures to Counteract Anoxia. Intermittent pressure breathing was first used at the Naval Medical Research Institute in the hope that the derangements of pulmonary blood flow associated with continuous pressure breathing could be circumvented. On an empirical basis it was found that a ratio of inspiratory to expiratory pressure of two to one with inspiration covering about two thirds of the respiratory cycle was highly effective. Useful consciousness was maintained at simulated altitudes of 47,000 feet for periods as long as 3 hours. The success of this type of breathing in our tests was due to the employment of denitrogenation for periods of at least 3 hours prior to pressure breathing at altitude.

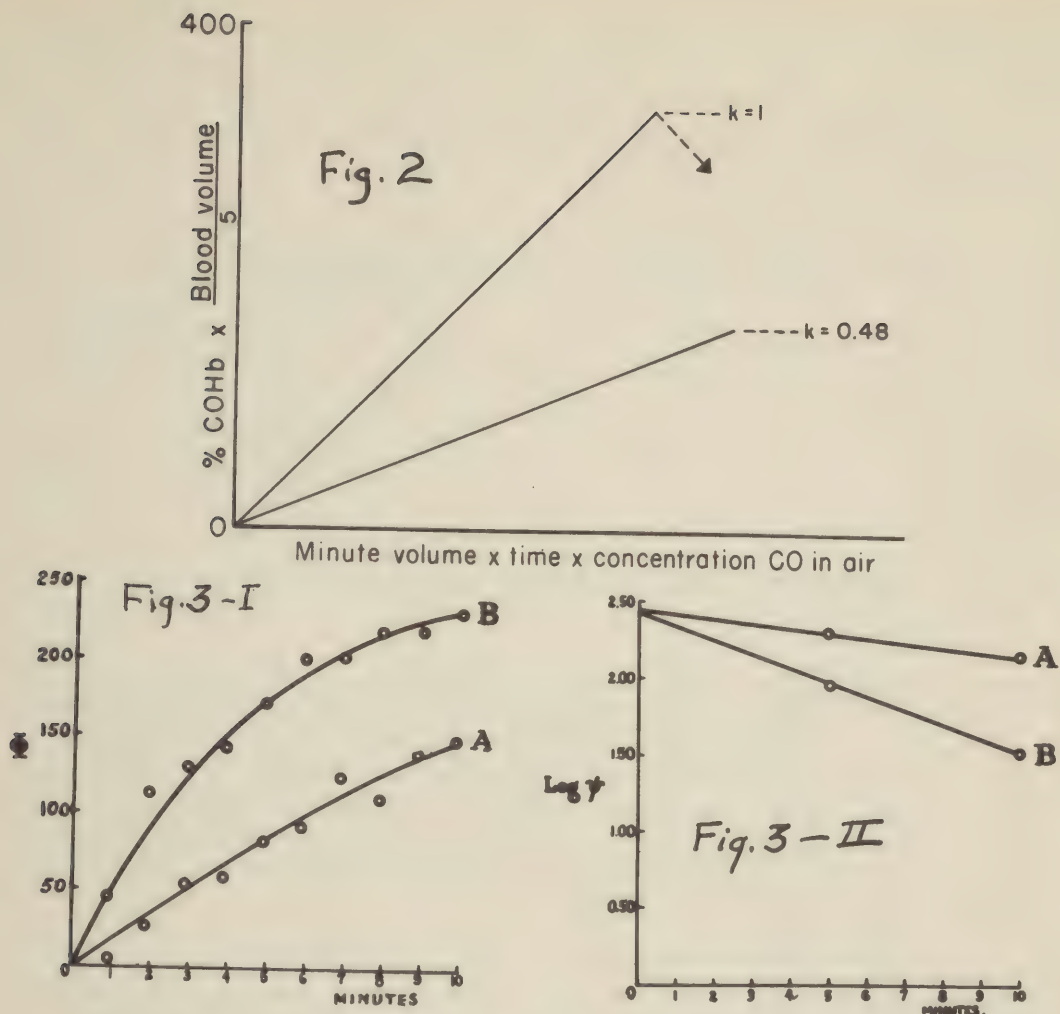


Fig. 2. Amount of CO taken up by the blood plotted against the quantity of CO inhaled. The slope of the line is governed by the fraction of CO taken out of the inhaled air. This relationship holds for a carboxyhemoglobin saturation of at least 30% (about 400 cc. CO).

Fig. 3. Absorption of inhaled radiokrypton by tissues of hand. I: A is normal curve of uptake of radiokrypton by resting individual during 10 minute period; ordinate represents counts of a Geiger counter held in the hand. B is the uptake of the gas in same resting individual following resumption of blood flow which had been cut off by means of a tourniquet on arm for 10 minutes. II: Semilogarithmic plot of same data. The slopes of A and B indicate relative rates of gas uptake.



Pressure breathing of air was employed to effect an increase by hyperventilation of the arterial oxygen saturation equivalent to air altitude gain of 7,000 feet. That hyperventilation and not pressure was the prime factor in raising the alveolar oxygen level was demonstrated by an experiment in the low pressure chamber utilizing the principle of the Drinker respirator.

#### Effect of Ventilation on Altitude Tolerance

Normal Respiration (Min.vol 8-10 liters/ min.)	Arterial Saturation %	Hyperventilation* (Min.vol 16-20 liters/ min.)
10,000 ft.	90	18,000 ft.
15,000 ft.	80	22,000 ft.
18,000 ft.	70	25,000 ft.

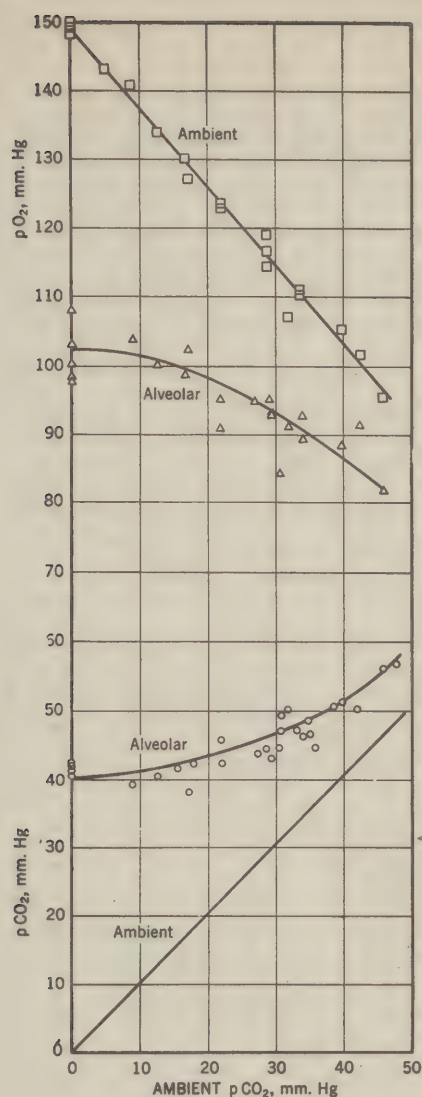
\*Thus, continuous experiment, 70 minutes at 18,000 feet, 15 minutes at 22,000 feet, and 15 minutes at 25,000 feet (31).

Injection of Red Blood Cells. A direct method of counteracting altitude anoxia and bringing about rapidly an apparent state of artificial acclimatization is the obvious technic of transfusing suspensions of red blood cells. By this procedure it was possible over a period of three weeks to increase significantly both the oxygen capacity and the oxygen content of blood. During the polycythemic period the pulse rate following exercise when breathing low oxygen mixtures was significantly lower than control measurements.

Carbon Monoxide Studies. The work of Haldane, Henderson, Drinker, and others has demonstrated not only the relationship between symptoms of carbon monoxide poisoning and the blood concentration of carbon monoxide, but also the quantitative nature of carbon monoxide uptake which makes possible a method for determination of blood volume. There remained, however, the problem of investigating the absorption of relatively high concentrations of CO over short periods of time. The chief contributions to aviation practices arising from these investigations were an accurate definition of, and a statement governing, the relationship between the variables involved in the carbon monoxide absorption for blood levels up to at least 30% COHb (fig. 2).

Effects of Prolonged Exposure to High Concentrations of CO<sub>2</sub>. The submarine submerged may be regarded as a metabolism chamber. Since the classic studies of DuBois and Brown following World War I, repeated measurements have shown an average hourly oxygen consumption of 0.9 cubic feet and a CO<sub>2</sub> output of 0.75 cubic ft. A matter of importance was the upper limit of CO<sub>2</sub> in conjunction with lowered oxygen content consistent with operating efficiency of submarine personnel.

In tests up to 72 hours duration, Consolazio and his co-workers demonstrated that exposure in CO<sub>2</sub> atmospheres up to 5% with a diminishing oxygen content as low as 12% did not impair the condition of



mm. Hg			
Ambient air		Alveolar air	
pCO <sub>2</sub>	pO <sub>2</sub>	pCO <sub>2</sub>	pO <sub>2</sub>
0.2	150.1	40.8	100.4
9.2	140.5	39.3	103.7
17.3	131.20	42.3	98.5
27.5	119.1	43.5	95.0
34.3	111.0	46.4	92.5
42.6	101.5	50.4	89.3
0.2	150.1	41.9	103.0
5.3	143.4	—	—
12.6	134.3	40.4	100.0
22.3	123.7	42.2	95.2
28.8	116.3	44.1	92.9
34.1	109.6	46.3	89.0
40.0	102.8	49.3	88.3
46.2	95.2	53.8	81.4
0.2	150.1	41.1	98.1
15.9	—	41.4	—
31.0	—	44.8	—
38.8	—	50.7	—
48.2	—	56.7	—
0.2	148.5	35.5	108.2
17.4	127.5	38.0	102.2
29.4	114.4	42.9	95.0
32.3	106.8	46.0	91.2
35.0	—	46.5	—
33.6	—	45.9	—
30.6	—	44.4	—
36.2	—	44.5	—
0.2	148.8	42.3	97.6
22.2	122.7	45.5	90.6
30.7	—	49.6	—
35.2	—	48.5	—

Fig. 4. Effects of inhaled CO<sub>2</sub> on concentrations of O<sub>2</sub> and CO<sub>2</sub> in alveolar air during course of experiments in which 500 cu. ft. of air per man was re-breathed. (Consolazio et al.).



personnel as evaluated by biochemical, physiological, and psychological tests. The increased pulmonary ventilation induced by breathing atmospheres rich in CO<sub>2</sub>, the increased circulation rate, and the more effective oxygenation of tissue served to render a 5% CO<sub>2</sub> -- 13% oxygen atmosphere roughly equivalent to a 3 to 17% atmosphere, heretofore considered to be the upper limit for prolonged exposure. That the 5% ambient CO<sub>2</sub> concentration is an upper limit for well being is apparent from figure 4.

It is observed that the body by increased respiration maintains a remarkably low alveolar CO<sub>2</sub> concentration despite the high ambient level. Above 5%, however, the CO<sub>2</sub> begins to rise in alveoli as it increases in the ambient air; the respiratory response has reached an upper limit for the maintenance of prolonged compensation.

Submarine Escape. The Momsen type lung was associated with the occasional development of excessive intrapulmonic pressures which ruptured pulmonary capillaries to produce air embolism.

Excess intrapulmonic pressures of the order of 80 mm Hg in dogs are accompanied by the appearance of gas bubbles in peripheral arteries. To obviate the difficulty of exhaling against hydrostatic pressure, Duffner is employing a hood which permits rapid ascent from depths of at least 150 feet without danger of building up excess intrapulmonic pressure since exhalation of air at the mouth level is now facilitated by the hydrostatic pressure.

Use of Radioisotopes of Inert Gases. The use of isotopes which emit gamma rays was developed by Lawrence, Jones and their co-workers to study the exchange of inert gases by the body or parts of the body, particularly the hand. Thus with proper shielding a Geiger counter held in the hand of an individual inhaling radiokrypton will measure the gamma ray emissions as the tracer gas diffuses into the tissues (fig. 3). From the results of Geiger counter measurements it was possible to plot the hand saturation curve for radiokrypton and to confirm the theoretical predictions of Smith and Morales regarding the shape and characteristics of this curve. These investigators used a summated series of exponential decay terms to describe gas uptake by an isolated region of the body as the hand in terms of such physiological parameters as blood flow, fluid and tissue cell volume, cell membrane permeability and the oil/water solubility of the solute. Their theoretical curve describing gas uptake by the hand is compared with experimental values.

Additional experiments with radiokrypton were also conducted to determine how the hand saturation rate was influenced by changing various physiological parameters. This if the blood supply to the hand is occluded for 10 minutes, the inhalation of radiokrypton reveals the effect of reactive hyperemia on gas absorption.

Protection Against Crash Injury and Blast Forces. An important part of the research program of the Naval Medical Research Institute is the investigation of methods for the protection of personnel against

crash injury, abrupt linear deceleration and impact forces. The problem involves a consideration of the limit of protection obtained by restraining devices, additional protection by energy absorbing devices and finally protection by precrash or crash ejection. The technics used by the mechanical engineer to measure the strength of structures under static and dynamic loads are being utilized to determine the effects of mechanical force on various tissues and organs.

In Draegers' study of the comminuted fractures of the ankle produced by underwater blast transmitted thru the steel hulls and decks of ships, it was found that protective devices comprising deformable metal loop heels and collapsible cellular plastic mats afforded sufficient protection to prevent such experimentally produced fractures. Thus if a metal loop is placed in the heel of a shoe, the compression of this loop through a distance of 1 inch may be sufficient to absorb the energy peak acceleration and to lower the impact force on the body itself to a level below the limit of tissue tolerance. If for example, a compression load of 1,000 pounds is necessary to break the tibia, the protective material used must undergo deformation under a lesser static load in order to be effective.

It becomes more and more apparent from the analyses of De Haven that the body is capable of withstanding tremendous forces if local deformation of tissue does not occur in vital areas. Thus in crashes of light airplanes it has been found that safety belts capable of resisting breakage up to 4,000 pounds and restraining an area of about 20 square inches at the hips have been broken in crashes with no apparent injury at the site of restraint or elsewhere.

In the experiments of Bierman impacts of 3300 pounds applied by means of a protective harness by dropping a 550 lb. weight at a distance of 6 inches and distributed over the therax and abdomen, were tolerated with little discomfort and with only such physiologic disturbances as temporary bradycardia.

Lt. Comdr. E. M. Wurzel (MC) USN, is formulating plans to study tissue tolerance and the physiologic effects to impacts by a dynamic rather than a static system consisting of a 500 foot tower in which test animals, volunteers and aircraft structure can be subjected to conditions obtaining in aircraft crashes.

Medical Aspects of Ionizing Radiations. Following the Atomic bomb explosions at Hiroshima and Nagasaki in August 1945, a field team organized at this Institute studied in detail the clinical and pathological aspects of the bombings, made measurements of the physical effects of the explosions, and assembled numerous data from various Japanese scientists. In the summer of 1946 in connection with OPERATION CROSSROADS key personnel from this Institute, utilizing an entire troop transport converted into a laboratory ship, made a quantitative assessment by means of hundreds of pigs and goats and thousands of small animals, of the probable effects of an atomic bomb explosion on personnel aboard naval ships. About 50% of the Bikini animals have been returned to this Institute where continued study of



the radiation effects is in progress. Some of the animals will be followed through their entire life span and their progeny studied to determine insofar as possible the effects of radiation on germ plasma. The hematologic changes are thus far the most sensitive indication of the injury produced by ionizing radiation.

Leucite Calvarium. The leucite calvarium preparation by Pudenz and Sheldon and later its improvement by Minard and Howell permits an inspection of the whole anterior surface of the cerebral hemispheres. This preparation was extremely useful in studying the mechanics of concussive forces, in observing the movement of gas bubbles through arteries and veins, and at Wright Field in studies of the effects of negative deceleration.

As currently used by Minard it is of value in studying various phases of cerebral circulation and circulatory time by means of fluorescein dye injected in peripheral vessels.

AERO MEDICAL EQUIPMENT LABORATORY, NAVAL  
AIR MATERIEL CENTER, PHILADELPHIA, PA.

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It seems to be open season on the general subject of comparison of research people in uniform and those not in uniform. Some of us in the Navy have been privileged to sit at the feet of civilian authorities in connection with Navy contracted research studies. Those of us who most enjoy meetings of this sort must attribute that understanding to the advantage we have had in learning research methods in civilian institutions. I believe that we of the military should seriously consider augmenting and fostering the concept of assigning some of the younger men in uniform to civilian laboratories to learn research methodology and scientific procedure.

I wish to refer to two generalizations which are being repeated almost daily in this country, particularly as viewed from my present administrative position.

Our laboratory, the Aero Medical Equipment Laboratory of the Naval Air Materiel Center in Philadelphia, enjoys a position which may be described as follows: When basic research as conducted by civilian laboratories or by the Navy Medical Research Institute reaches a stage in which the results should have validation in large population groups, they are referred to Pensacola. When they involve flight testing, they go to Patuxent. If conclusions seem to warrant their inclusion into aviation and when it is desired to know if principles will work for aviators in airplanes, the problem comes to our laboratory. Our position is to receive suggestions and tentative conclusions which come from basic research to determine whether or not they will work in airplanes and our projects are almost all of this nature. Our work is intimately coordinated with engineers in other laboratories of the Experimental Station.

I wish to amplify previous statements regarding the need for increased fundamental research. Reference to the need occurs almost daily. The Steelman report to the President on Manpower for Research has that as an outstanding recommendation. There is a crying need for basic, fundamental research. This merely augments what we are all aware of, but I give it emphasis. The second generalization I would like to emphasize is the difficulty of drawing a line between "pure" research and applied development. It is extremely difficult. I would



like to illustrate these points as they appear in three projects in our laboratory.

The first is the problem of forceful ejection from high performance aircraft. We received the Martin-Baker test tower and seats from Great Britain for a general evaluation to see if they would work and if seat ejection could be perfected. It became apparent that the early German work had been very poorly instrumented and documented and, as there had not been time for Great Britain to conduct these tests, there were few data on exactly what goes on in the human body during high accelerations of short duration. It was necessary for us, as a purely developmental laboratory, to go back to the most fundamental kind of research. It was necessary for us to develop basic data in order to extend them into practical development. This illustrates the difficulty in separating the pure from the applied or developmental research.

Another subject is our current problem on the effects of vibration on the body. In our laboratory we have a unique situation in that we are presented with a test situation which is unusual and over which we have very little control. We have jet engines running in enclosed test cells. Our problem is to determine the physiological effects of the operation of the jet engines in a predetermined experimental setup. We have encountered the problem of the vibration spectrum. The physicists tell us that they do not have equipment to make determinations of the spectrum to the degree of precision they demand. So we do not know what the spectrum is and it will be some time before we do. If basic research had provided this equipment our task would have been materially eased. It would be desirable from the scientific point of view, if we could first set up a very precise vibration band, control intensities, expose experimental animals, and find what the responses are to specific bands. If we knew reactions to specific bands in the spectrum, we would be better able to continue our determinations. There should be accelerated efforts to find these effects.

The ventilation mechanisms in high performance airplanes are being studied and we are presented with estimations such as skin temperatures on the planes being 1,500 degrees at certain high speeds. In the consideration of heat exchange and comfort levels in certain temperature and humidity situations, we have become accustomed to use well-accepted formulas to make our determinations. But when we consider these unusually severe conditions, we find those formulas can no longer be used and we will have to conduct some very basic fundamental research to adjust these formulas to fit the situation.

I would like to conclude with a statement which is probably more philosophical than physiological. That is to express the need for changing our attitude toward tolerance maxima from the physiological point of view. Some years ago we were given to making certain categorical statements as to what tolerances were. We said for example, that 30,000 feet was the human ceiling even with pure oxygen. We stated certain tolerance limits of acceleration, etc. In many instances we have had to eat our words. We find, in the determination of maximum

tolerances, a shift in our attitude. People often say, "Why don't you give up this will-o'-the-wisp search for methods of making it possible for people to fly these things?" "All military flying will be in guided missiles anyway." But even if we are going to have guided missiles, somebody will have to be close enough to guide these missiles. We propose not to give up to that line of reasoning. We propose to go on developing equipment and are confident that aviation medicine will be able to keep pace with the engineers in their progress. When asked in regard to seat ejection to express the maximum tolerances a man can stand, we reply that we don't know. We prefer to reason as follows: We have developed an ejectable seat which can be shot out of an airplane by a powder charge in the catapult to a height that will adequately clear any existing or planned airplane. In doing so we develop accelerations within the pilots body of 18 to 20 G. We have demonstrated by several hundred tower experiments, using both dummies and human subjects, that, if these accelerations are not initiated at a rate of more than 200 G per second and if the dynamic response factor is not greatly in excess of 1.2 these accelerations are well within human tolerable limits. We intend to postpone the day when we must say to engineering development, "Thou shalt go no further!"





# ESTIMATION OF CRITICAL DEAD SPACE IN RESPIRATORY PROTECTIVE DEVICES<sup>1</sup>

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Increasing external dead space is an inevitable accompaniment to the use of various respiratory protective devices. Frequently minimal dead space may not be compatible with other essential features of design. The data to be presented in this paper were gathered in response to the practical need for estimation of maximal external dead space consistent with "normal" respiratory behavior. They illustrate some of the problems met continuously in all branches of military physiology and, in addition, present some unexpected physiological relationships.

Method. A closed circuit, dry, double tube metabolism apparatus (McKesson "Metabolor") was employed for recording tidal volume, respiratory frequency, ventilation rate, and oxygen consumption. The amount of rebreathing was varied by insertion of rubber tubing, of bore commonly employed in oxygen equipment, between the subject and recording apparatus. Respiratory pressures were measured by a water manometer attached to the mask and are expressed as average maximal pressures over each test period.

When desired the Pauling oxygen tensimeter was connected into the system and samples of gas circulated through it by means of a small pump.

All tests were made at ground level pressure. The 7 subjects were adult males, tested  $1\frac{1}{2}$  hours postcibal. Three 8 minute control periods were followed by several test periods with added dead space and, when desired, the control values redetermined. Alveolar samples were collected after forced expiration at the end of each period. Tests were arranged to assure steady state conditions and data indicating deviations from the steady state were discarded.

Owing to the length of the experiments a comfortable mask was substituted for the conventional mouthpiece. Comparisons were made

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<sup>1</sup> Part of the material in this paper was contained in a report circulated by the Committee on Aviation Medicine, National Research Council as Report No. CAM 238, dated January 1, 1944.

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between the mask alone and mask plus additional tubing. An additional series was run to compare the effects of the mask (approximately 100 ml) and the mouthpiece. No significant differences were observed in this latter case, but the experimental comparisons must be considered as from a base line of 100 ml external dead space.

Exercise, where present, consisted of pedalling a stationary bicycle at a rate and load sufficient to double the rate of oxygen consumption over the sitting-rest value.

Statistical significance of the effects was determined by application of the Fisher "t" test (1) and in one case by calculation of the fiducial limits of the mean<sup>3</sup>.

Results. Table 1 illustrates the effect of various added dead spaces up to 540 ml on alveolar pCO<sub>2</sub>, tidal volume, and respiration rate. Except for one value it is seen that alveolar pCO<sub>2</sub> does not change significantly under resting conditions until an added dead space of 540 ml is reached; under exercise conditions a significant rise occurs at 320 ml. Significant changes in tidal volume appear almost immediately on adding dead space while respiration rate shows no change of significance except with the largest volume under exercise conditions. Not shown in the table is the fact that oxygen consumption showed no change in any case.

Table 1. Change in Average Alveolar pCO<sub>2</sub>, Tidal Volume, and Respiration Rate on Adding Various External Dead Spaces.

Dead Space Added <sup>2</sup>	Alveolar pCO <sub>2</sub>		Tidal Volume <sup>3</sup>		Respiration Rate	
	Rest	Exercise	Rest	Exercise	Rest	Exercise
ml	mm Hg	mm Hg	ml	ml	Resp/min	Resp/min
150	2.0 <sup>1</sup>	0.1 <sup>1</sup>	132	6 <sup>1</sup>	-0.3 <sup>1</sup>	-0.3 <sup>1</sup>
250	3.0	2.1 <sup>1</sup>	209	111	0.6 <sup>1</sup>	-0.4 <sup>1</sup>
250 <sup>4</sup>	1.2 <sup>1</sup>	—	163	—	1.0 <sup>1</sup>	—
320 <sup>4</sup>	0.9 <sup>1</sup>	4.3	144 <sup>1</sup>	114	1.5 <sup>1</sup>	-0.3 <sup>1</sup>
450 <sup>4</sup>	2.2 <sup>1</sup>	4.0	347	310	0.3 <sup>1</sup>	0
540 <sup>4</sup>	5.6	4.3	410	386	-0.3 <sup>1</sup>	-1.5

<sup>1</sup> Change not significant statistically (Fisher "t" test, p value 0.01)

<sup>2</sup> Total dead space 100 ml greater

<sup>3</sup> STP dry

<sup>4</sup> 3/4 in. bore, others 5/8 in. bore

<sup>3</sup> Thanks are due to Dr. M. R. Zelle for this latter calculation.

Figure 1 presents the alterations in rate of pulmonary ventilation under the same conditions. All increases are significant statistically except the first point in the presence of exercise. This figure shows that changes in pulmonary ventilation occur with only small increments in dead space, and, combined with the data contained in table 1 show that the increase in ventilation is accomplished largely by increasing the tidal volume rather than the respiration rate.

An unexpected feature of the data in figure 1 is the fact that the increases in pulmonary ventilation were considerably smaller in the presence of light exercise than under resting conditions. This is true on both an absolute and on a percentage basis. The limits of the range of expected variation of any given observation from the mean are indicated in the figure (calculated as fiducial limits). The lessened response under exercise conditions is clearly significant.

Table 2 presents the changes in respiratory pressures occurring on addition of dead space. The shape of the space was chosen to simulate current oxygen supply equipment practices. While small, these changes are significant statistically in the majority of instances. Changes in resistance to breathing were subjectively the most noticeable element of difference among various tubes. In fact, subjective response followed more closely the pressure patterns than any of the other parameters. Not shown in the tables are readings for a tube with 250 ml volume but fitted with a restriction causing about 1 cm water further increase in pressure drop. This tube was subjectively less comfortable than any but the 540 ml volume in spite of the fact that ventilation rate was considerably higher with the latter tube.

Experiments in which oxygen tension was measured indicated that this was maintained constant in both the mask and alveoli until alveolar  $p\text{CO}_2$  began to rise, in which case  $p\text{O}_2$  fell in proportion to the rise in  $p\text{CO}_2$ . Statistical validity of changes in  $p\text{O}_2$  was not tested.

Discussion. Physiological effects of added external dead space are related principally to the increased volume for rebreathing and the increased resistance to gas flow. The data presented above corroborate the opinion that subjectively the resistance factor will likely outweigh the rebreathing factor as bulk is increased, especially with equipment of current design. The data also indicate that dead space might be increased safely in certain instances to reduce resistance.

It is clear that increases in pulmonary ventilation prevent a rise in alveolar  $p\text{CO}_2$  under resting conditions until fairly large dead spaces are added. In the presence of light exercise the addition of dead space is not met by as large increases in ventilation as at rest, possibly because of the smaller ratio of the added dead space to the



tidal volume. This results in significant rises in alveolar  $p\text{CO}_2$  at smaller added dead space than under sitting-rest conditions.

Table 2. Effect of Added External Dead Space on Respiratory Pressures

Dead Space Added <sup>2</sup>	Bore	Increase in Maximal Inspiratory Pressures <sup>3</sup>		Increase in Maximal Expiratory Pressures <sup>3</sup>	
		Rest	Exercise	Rest	Exercise
ml	in.	cm water	cm water	cm water	cm water
150	5/8	0.4 <sup>1</sup>	1.8	0.7 <sup>1</sup>	0.9
250	5/8	1.2	1.9	1.1 <sup>1</sup>	2.2
250	3/4	1.4	---	0.5	---
320	3/4	1.1 <sup>1</sup>	1.4	0.8 <sup>1</sup>	1.2
450	3/4	1.0	2.4	1.6	1.9
540	5/8	3.3	5.3	4.8	3.8

<sup>1</sup> Change not significant statistically (Fisher "t" test, p value 0.01)

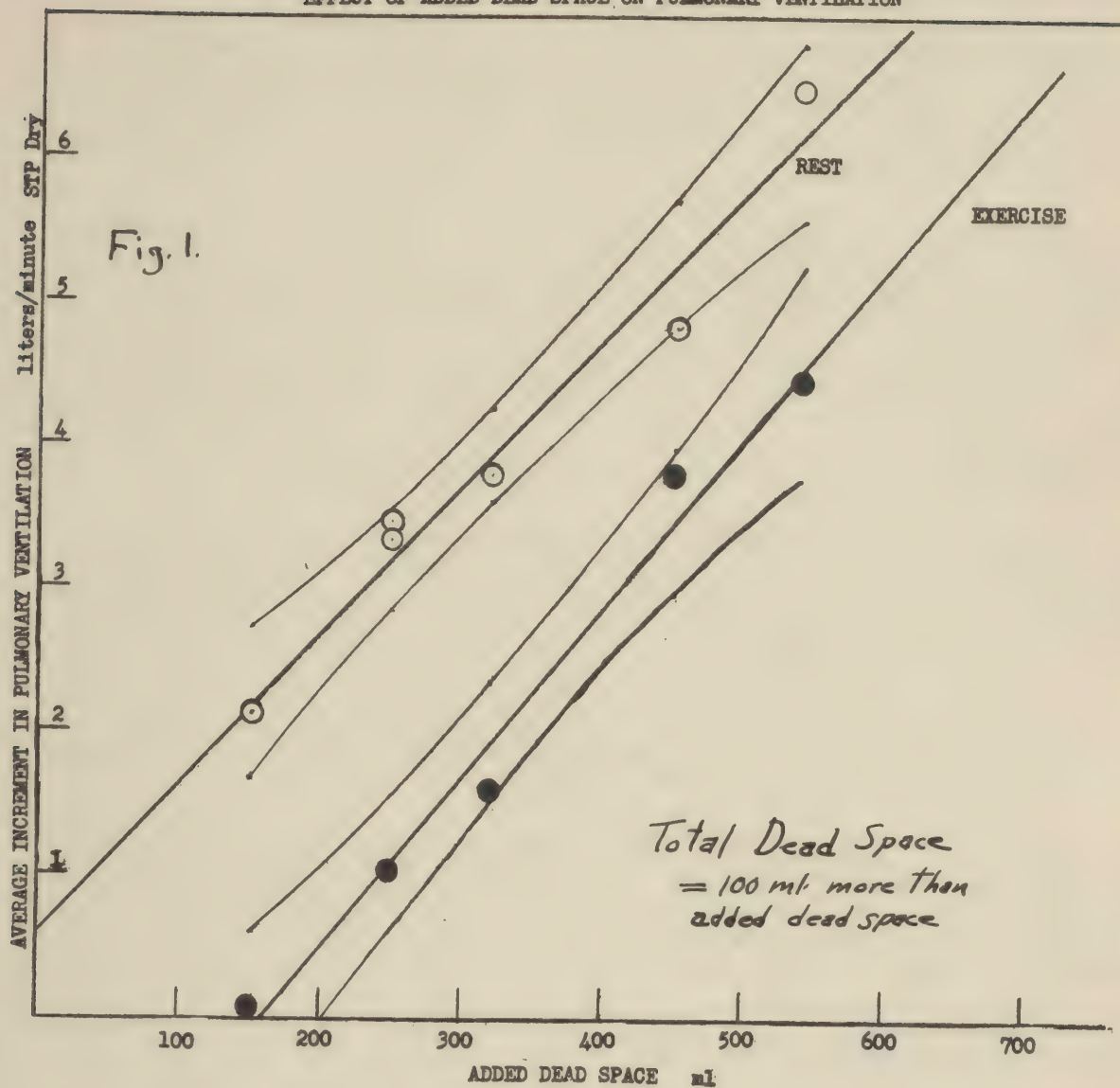
<sup>2</sup> Total dead space 100 ml greater

<sup>3</sup> Average maximal pressures

Studies complementary to the present one have appeared in the Italian literature (2, 3). Tomasso (2) exercised his subjects actively at ground level while Margaria (3) determined the effect of added external dead space on altitude tolerance. These investigators did not test the statistical validity of their results but it is clear in both instances that alveolar  $p\text{CO}_2$  remains constant up to added dead spaces approximating 300-350 ml. Margaria's results show that at altitude (7,000 meters) alveolar  $p\text{O}_2$  is influenced by dead space only when ventilation is insufficient to prevent a rise in alveolar  $p\text{CO}_2$ .

Data on pulmonary ventilation and alveolar  $p\text{CO}_2$  at dead spaces between 20 and 1575 ml have been obtained by Otis, *et al.* (4) and Fenn, *et al.* (5), for other purposes. Their results indicate a rapid rise of pulmonary ventilation and alveolar  $p\text{CO}_2$  at dead spaces higher than those employed in the present study. Alveolar  $p\text{CO}_2$  values for subjects breathing air at 16,000 ft. (5) show that hypocapnia may, under these conditions, modify the effect of the smaller dead spaces on ventilation and  $p\text{CO}_2$  at least until equilibrium conditions can be established.

# EFFECT OF ADDED DEAD SPACE ON PULMONARY VENTILATION





The effect on pulmonary ventilation of the dead space contained in the A-13 and A-14 oxygen masks and of various lengths of tubing added thereto was determined by Hall, Wilson, and Dahling (6) under conditions of rest and light exercise. Their report appeared in the military literature just prior to our preliminary report. The difference in ventilation increment noted in the data presented in figure 1 above is not as apparent in their studies. This may be attributed in part to the relatively short range of dead spaces added but other variables may have contributed.

Estimation of a "critical dead space" is governed by the criterion chosen. No volume tested above 100 ml could be considered without effect on pulmonary ventilation as seen in both the present and other studies. If maintenance of constant and nearly normal alveolar CO<sub>2</sub> and O<sub>2</sub> tensions is sufficient the critical total volumes would approximate 400 ml under exercise conditions and 600 ml under resting conditions. These values may be utilized as a working hypothesis but the increased respiratory effort necessary may involve fatigue factors of importance in designing apparatus for long periods of use.

Summary. Effects of added external dead space at sea level pressure have been determined in tidal volume, respiration rate, pulmonary ventilation rate, alveolar pCO<sub>2</sub>, oxygen consumption, and inspiratory and expiratory pressures. Total dead space volumes ranged from 100 ml to 640 ml, with increments varying from 150 to 540 ml.

Pulmonary ventilation and tidal volume were found to be the most sensitive functions by objective measurement while changes in respiratory pressures were the most sensitive subjectively.

Ventilation rate responded less adequately to increase in dead space when the subject engaged in light exercise than under sitting-rest conditions. The increases were smaller on both an absolute and relative basis, and as a result, alveolar pCO<sub>2</sub> rose at lower dead spaces under exercise conditions.

Changes in respiration rate and oxygen consumption rate were negligible under the conditions of the study.

"Critical" dead space depends upon the criterion chosen. All additions above 100 ml had some effect, but if maintenance of alveolar pCO<sub>2</sub> is chosen as the criterion of safety, critical spaces may be estimated as approximately 600 ml at rest and 400 ml under light exercise conditions.

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SOME EFFECTS OF CHANGES IN THE DENSITY OF RESPIRED  
GASES ON THE VELOCITY PATTERNS OF THE BREATH

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In the course of assessing oxygen supply equipment for Navy air-crews observations were made on the behavior of the lung ventilation with regard to tidal volume and inspiratory and expiratory intervals. These data indicated that, even under conditions of full oxygenation, these phenomena undergo some changes when the absolute pressure is decreased from about 760 to 225 mm Hg (30,000 ft.). A series of experiments was set up subsequently to determine quantitatively on a larger group of subjects the magnitude of these changes in the ventilatory act.

In order to interfere as little as possible with resistance factors and to obtain data on both inspiratory and expiratory phases in a continuous record, a respiratory flowmeter was constructed on the principle developed by Leslie Silverman at the Harvard Fatigue Laboratory (1). Modifications were made in this basic design partly by choice and partly by the limitations in available instrument components at the time.

The apparatus design induced some conditions in the experimental setup which, although not desirable, were judged necessary and were held as constant as possible. The most significant change from normal conditions was an increase in the external deadspace due to the necessity of rebreathing through the flowmeter. The effect on the ventilatory act is recognized and some estimate of the nature and extent of such an effect can be made, particularly in view of the work done by Dr. Stannard on this subject in our laboratory.

In the past, greater emphasis has been laid on the assessment of the mechanical condition of the lung tissues and the activity of the respiratory reflexes (2) than on the relation of air velocities to factors of density and viscosity and to the dimensions of the respiratory passages. This subject was included in studies by Dean and Visscher (3) in the analysis of reported clinical benefits from the use of helium-oxygen mixtures in the treatment of status asthmaticus and similar conditions in the lungs. Their analysis was complicated by the fact that a change in both density and viscosity is effected by the addition of helium to oxygen, and further by the fact that in this mixture density falls while viscosity rises. They were, thus, not in a position to make an independent check on the effect of either factor separately and relied on certain assumptions concerning the dimensions of the air paths and the estimated velocities of the gases in the lung to conclude that density per se was the effective factor in the relief afforded by the therapeutic use of helium-oxygen mixtures.

Our approach to this subject has been from the point of view that by simulated ascent a decrease in the statistical density and weight of the



respired gases can be effected without change in the viscosity, since in the pressure range used there is no appreciable effect on the viscosity of gases. Thus rarefaction of the atmosphere would be expected to facilitate the volumetric exchange of gases in the lungs provided that the condition of full oxygenation is maintained.

We have now completed work on breath pattern records on 32 young men taken to 30,000 ft. altitude under various conditions and have under analysis breath pattern records of another group of 12 men tested with helium-oxygen mixtures. The major effects are seen in alterations in the velocity of the breath, particularly in expiration, and in the concomitant timing of the phases of the breath cycle. While a very detailed analysis of the breath velocity pattern was made in reading the records of each individual I will limit my discussion to those factors showing changes of a significant character. One of the phases of this work which at this time cannot be properly assessed comprises the variations from one individual to another within the so-called normal range. We hope to be able to treat this more fully when frank pathology can be analyzed according to the same criteria that were used on these subjects.

The subjects were given our test seated at rest and again following an exercise consisting of fifteen deep knee bends. An arbitrary restriction of the inspiratory duct was also made under the same conditions to attempt an estimate of the effect of unavoidable resistance in the apparatus on the velocity pattern of the breath.

The most significant changes observed under conditions of rest by virtue of the rarefaction of the atmosphere are a marked lengthening of the post-expiratory pause (167%), and increase in the maximum expiratory velocity (16%), an increase in the initial acceleration of expiration, and decreases in tidal and minute volume (10 and 12% respectively). All other factors also indicate a hastening of the execution of the ventilatory act, although to a less significant magnitude.

It is well to point out here that these are average values and thus mask the individual responses, which may vary not only in magnitude but also in sign. In this instance it was observed that the expiratory pause was lengthened in those individuals who demonstrated it at ground level while among those that did not show a pause at ground level, there were some who interposed a pause while at altitude. Thus, the net result was an increase in the length of this average value after ascent.

Following exercise the changes induced by rarefaction are such as to facilitate all accelerations and thus to permit a greater tidal and minute volume. This increased ventilation is accompanied by an increased breath interval, mainly by virtue of the addition of pauses. This was a rather unsuspected development in view of the usual concept that pauses are quickly lost in the ventilation following exercise.

The presence of restriction in the inspiratory duct tends to increase the differences produced by rarefaction of the atmosphere. It is possible to ascribe this to the effect of resistance on the ground level situation in that pauses and the breath cycle are shortened by such restriction. On the other hand, following exercise, rarefaction of the atmosphere did not



permit the interposition of pauses. It is possible that varying pressure or proprioceptor stimuli are responsible for these variations in behavior and any further experimentation must include such data for adequate analysis.

If we consider, for the sake of discussion, that in each of the various conditions, carbon dioxide production is relatively uniform, then the timing of the breath cycle is dependent on the rate of carbon dioxide loss from the lung. If this loss reduces the partial pressure of carbon dioxide in the blood within each cycle so that it is still below the threshold for initiating inspiration then a pause should be interposed between cycles.

The demonstration of significant increases and de novo interposition of pauses in the breath cycle after ascent is consistent with a facilitation in the efficiency of removal of carbon dioxide from the circulating blood due to a decrease in density.

The effect of helium-oxygen mixtures as compared to that of rarefaction of the atmosphere is about one-half at equal density conditions. This has been investigated in a preliminary series of experiments the data of which were obtained on a different group of individuals than those on which the altitude tests were made and are thus not conclusive. We are now in the process of analyzing further experimental data of both helium-oxygen and altitude conditions compared on the same subjects and within a short time interval.

From the preliminary data on helium-oxygen mixtures we have been able to discern what may be an effect of viscosity in the breath pattern. This has shown itself in the marked reduction of initial accelerations of the breath flow at rest, a situation in which the viscous factor should be more effective than any of the other criteria used. It does not appear in exercise. Further analyses should clarify the relation between these two factors and indicate the reliance which may be placed in such criteria.

From a theoretical point of view this work is of interest because new methods of assay of lung function may be developed. It is possible to look upon the distribution of ventilative ability among individuals as ranging from the condition in which pauses are interpolated normally in consequence of efficient exchange of gases, down through the condition of bare adequacy, to that in which compensations of another character must be called into play. It may be possible by alteration of the density in one way or another to bring in or suppress breath pauses. Density will then become an index of the ventilative efficiency of the lung system. One rather important advantage of such a method would be the minimizing of subjective effects which is at present a serious difficulty with such investigations. If the quantitative relation of viscosity and density is thoroughly established then it should be possible to use gas mixtures for density changes rather than rarefaction and compression, with their attendant hazards.



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CHANGES IN ALVEOLAR GAS TENSIONS DURING  
ACCLIMATIZATION AT 10,000 FEET AND THE EFFECT  
OF THE LATTER ON ALTITUDE TOLERANCE

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To date, flying personnel have not been exposed to altitudes in planes long enough to produce any degree of the kind of adaptation to altitude one may call acclimatization. Their physiological responses fall into the category better referred to as accommodation: such reactions as increased ventilation, increased heart rate, and other short-term functional responses. The long-term, organic changes, such as the increase in red cell count, increased hemoglobin levels, etc., do not occur.

Recently, however, as planes have pushed higher into the stratosphere, the question of man's ability to survive even when breathing 100% oxygen has become a prime problem. Ordinary pressure breathing will add several thousand feet to the physiological ceiling, and pressure cabins might make any distance above the earth's surface attainable; but from the practical point of view, these means may under some circumstances be undesirable. In war, for example, the hazard of explosive decompression may limit the use of the pressurized cabin. Consequently, if a little higher ceiling, or a little more time at ceiling can be gained in some other way the advantage might be of inestimable tactical value.

For that reason, the possibility of using acclimatization as a means of augmenting anoxia tolerance has been investigated. The correlated changes in alveolar gas tensions have likewise been observed. Twenty-seven subjects ranging in age from 18 to 40 years were assembled at Randolph Field for baseline studies of various functions. These included determinations of the  $pO_2$  and  $pCO_2$  in alveolar air samples, analyzed by the Peters and Van Slyke modification of Haldane's method. Tests for anoxia tolerance were done by measuring the duration of "useful consciousness" at a simulated altitude of 25,000 feet in a low pressure chamber.

At the end of the standardization period the subjects were transported from Randolph Field (elevation 750 feet) to Leadville, Colorado (elevation 10,200 feet). The comparative observed barometric pressures and calculated oxygen tensions in inspired air at the two places are shown in figure 1. The oxygen tension in inspired air is at Leadville about  $2/3$  of that to which the subjects were accustomed.



The group remained at Leadville 2 weeks, during which time the laboratory studies of alveolar air were continued. On the hypothesis that acclimatization would proceed more rapidly if the subjects were physically active, participation in sports, hiking and mountain climbing was encouraged. At the end of the 2-week period all subjects returned to Randolph Field, where testing was immediately resumed, and altitude tolerance was again determined in the low pressure chamber.

Results. The results are shown in figure 2. At Randolph Field alveolar oxygen tensions averaged about 95 mm Hg, and the CO<sub>2</sub> tension 43 mm Hg. One day was spent travelling to Leadville and setting up apparatus. By the morning of the next day, Day No. 1 of the operation, the alveolar pO<sub>2</sub> had fallen to an average of 55.8 mm Hg and the pCO<sub>2</sub> to 35.5 mm Hg.

During the ensuing 2 weeks the alveolar pO<sub>2</sub> rose gradually but irregularly to 59.6 mm Hg tension, and the pCO<sub>2</sub> fell slightly further to 33.8 mm Hg. On the 14th day the group returned, and on the 15th day (Day No. 1 at Randolph Field) testing showed that alveolar oxygen and carbon dioxide tensions had both risen. Alveolar pO<sub>2</sub> had even surpassed the pre-Leadville figure, averaging about 98 mm Hg tension. Repeated analyses showed that the oxygen tension did not completely and permanently return to its previous value for almost 3 weeks. Carbon dioxide tension, on the other hand, did not attain its pre-Leadville value that first day, rising to only 37.5 mm Hg. Approximately 2 weeks passed before a figure of 41 mm Hg was reached, and here it leveled off.

In the upper part of figure 2 are shown the results of the tests for duration of "useful consciousness." There was produced by the stay at 10,000 feet altitude a definite improvement in anoxia tolerance, amounting on the average to more than twice the pre-acclimatization interval of consciousness (11 minutes as compared to 5). In some cases the interval was even tripled. This increased tolerance was, however, only transitory. At the end of three weeks approximately half of the advantage gained had been lost (average duration down to 8 minutes), and at the end of two months the group average had decreased to only 7 minutes.

Fig. 1

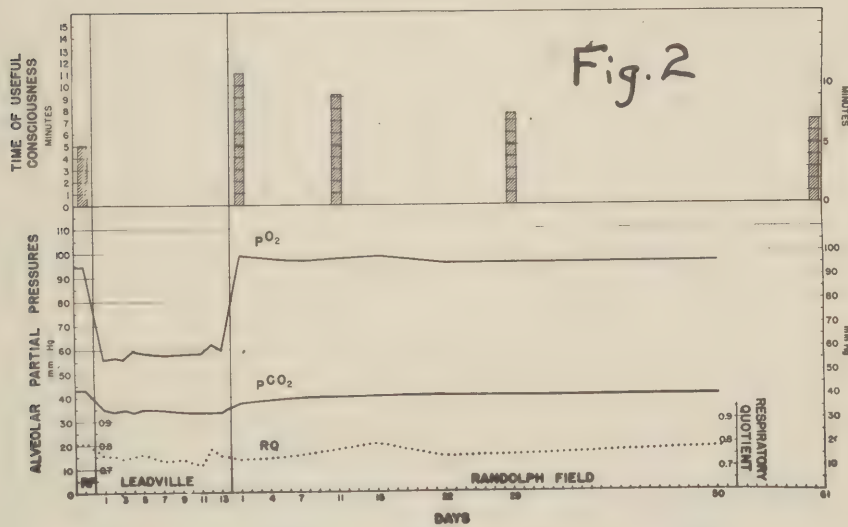
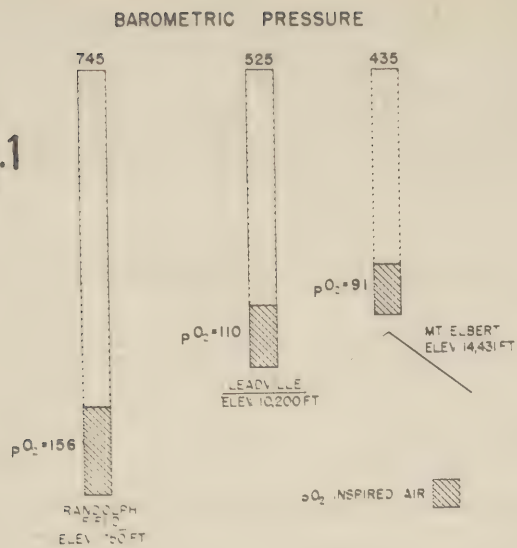


Fig. 1. Comparison of barometric pressures and oxygen tensions in inspired air at the several altitudes to which the group was exposed.

Fig. 2. Changes in alveolar oxygen and carbon dioxide tensions, R.Q., and "times of useful consciousness" at 25,000 feet simulated altitude.



It is of some interest to note that when the alveolar O<sub>2</sub> and CO<sub>2</sub> tensions are inserted into Dr. John Gray's alveolar equation, and the R.Q. calculated by this method, the group as a whole evidently displayed some lowering of R.Q. while at Leadville. This calculated "alveolar R.Q." is plotted in figure 2, where it will be seen to run consistently in the neighborhood of 0.75. Nor did it return immediately to normal values on return to Randolph Field, remaining somewhat depressed for several weeks.

Table 1. Alveolar O<sub>2</sub> and CO<sub>2</sub> tensions at 10,000 Feet Altitude

Source of data	Conditions	Alv. pO <sub>2</sub>	Alv. PCO <sub>2</sub>	R.Q. Calculated from Gray's Alveolar Equation(1)
Boothby(2) Lutz & Schneider(3)	Acute exposure in low pres- sure chamber	61	35.5	.89
SAM	Acclimatiza- tion for two weeks	59.6	33.8	.75
Fitz- Gerald(4)	Acclimatiza- tion for one year	65	30	.83

The degree of "alveolar acclimatization" which had been attained in the 2-week period may to some extent be judged by comparing the alveolar gas tensions both with those obtained during acute exposure in the low pressure chamber, and with those determined in fully acclimatized persons. This is done in table 1. There it is shown that in so far as alveolar CO<sub>2</sub> tensions are concerned the group had progressed in 2 weeks practically half way to complete acclimatization. The alveolar oxygen tensions are equivocal, possibly by reason of a lowered R.Q.

In so far as increase of ventilation is concerned, table 2 shows that the group had effectively improved its ventilation rate compared to acutely exposed individuals. These rates are calculated from the proportion to which alveolar CO<sub>2</sub> tension was decreased.

Rahn, Otis, and Fenn(5) have suggested that acclimatization produces an increase in sensitivity of the respiratory center to CO<sub>2</sub>. This appears to be a very likely possibility in view of, a, the gradual improvement of O<sub>2</sub> tension and of ventilation with the prolongation of

the stay at altitude, and, b, the persistence of a decreased alveolar pCO<sub>2</sub> together with a raised pO<sub>2</sub> after the return to an elevation where anoxia could no longer have been a stimulating factor.

Table 2. Ventilation produced by Exposure to 10,000 feet  
Altitude Compared to Ventilation at Ground Level

Source of data	Conditions	Alv. pCO <sub>2</sub> Ground Level	Alv. pCO <sub>2</sub> 10,000 feet	% Increase Ventilation at 10,000 Ft*
Boothby(2); Lutz & Schneider(3)	Acute exposure in low pres- sure chamber	40	35.5	113%
SAM	Acclimatiza- tion for two weeks	41	33.8	121%
Fitz- Gerald(4)	Acclimatiza- tion for one year	40	30	133%

\*  $\frac{\text{Alv. pCO}_2 \text{ ground level}}{\text{Alv. pCO}_2 \text{ 10,000 feet}} \times 100 = \% \text{ increase of ventilation}$

Summary. The feasibility of producing in Air Force personnel by means of a comparatively short stay at a moderate mountain elevation a worth-while increase in anoxia tolerance seems to be well established by the results of this operation. "Time of useful consciousness" at 25,000 feet altitude was more than doubled; alveolar pO<sub>2</sub> and pCO<sub>2</sub> were well on their way to acclimatization levels; and some confirmation of the suggestion that respiratory sensitivity to CO<sub>2</sub> is increased was obtained. The degree of acclimatization produced had practically disappeared in about 2 months.





## OXYGEN DIFFUSION AND TRANSPORT IN EXPLOSIVE DECOMPRESSION

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Prompted by the development of pressure-cabin aircraft during the last 10 years a considerable amount of study in aviation medicine has been directed to the effects of rapid decompression. Thus far the main interest has been focused on the immediate mechanical effects of extreme and sudden reduction of the total barometric pressure on living organisms, such as expansion of internal body gases, formation of gas bubbles in blood vessels, cerebrospinal fluid and tissues. These investigations were essential in the initial stages of research in a field which was very little explored and theoretically very dangerous. Fortunately the dangers of explosive decompression have been substantiated only to a limited degree. Based on experimental data available today in this country and elsewhere it is safe to say that an instantaneous drop in ambient pressure from 1 atmosphere to 1/10 of an atmosphere and less within fractions of a second may be injurious but not necessarily fatal.

In view of the foregoing, the real dangers of explosive decompression will result from the subsequent lack of oxygen rather than the mechanical effects per se. Measures taken for the survival and revival of aircrews in distress during stratosphere flight must be based on an exact knowledge of the particular type of hypoxia induced by explosive decompression. At the same time we were attracted by the prospect of gathering information of general interest on the movements of respiratory gases in the lungs and blood under these conditions. A number of investigations have been carried out by Clamann<sup>(1)</sup>, Lutz<sup>(2)</sup>, and Gelfan<sup>(3)</sup> on small animals with a view to ascertaining the survival-time on exposure to instantaneous decompression at various altitudes. With increasing altitude survival-time decreases but reaches a minimum which remains constant independent of altitude<sup>(3)</sup>.

In our preliminary experiments, constant survival-time was reached at approximately 53,000 feet (75 mm Hg) when the animals breathed oxygen. Using air the constant survival-time was reached at 44,000 feet (116 mm Hg). This was quite a surprise because we were willing to believe that at 53,000 feet breathing oxygen a state of complete anoxia is reached as the sum of carbon dioxide and water vapor pressure may equal the total pressure in the lungs. According to the familiar concept of "equivalent altitudes", however, we were led to expect the minimum survival-time breathing air at a much lower altitude than was determined by experiment. The application of the alveolar equation to



compute equivalent altitudes breathing air and oxygen is not possible under these circumstances as the necessary assumptions are valid only in a respiratory steady state. But it is permissible to compare the altitudes in question on the basis of tracheal oxygen pressure.

$$pO_2\text{trach} = (B-47) F_{O_2}(\text{insp.})$$

B=total barometric pressure  
 $F_{O_2}$ =fraction of oxygen(inspired air)  
 47 mm=PH<sub>2</sub>O at body temperature

Any major discrepancy in physiological response at equal oxygen pressure in the inspired air would imply different conditions of gas exchange in the alveoli. The altitude of "absolute hypoxia" (minimum survival-time) was determined at 53,000 feet with oxygen. The tracheal oxygen pressure is

$$pO_2\text{trach} = (75-47) 1.0 = 28 \text{ mm Hg}$$

The equivalent altitude breathing air with identical tracheal pO<sub>2</sub> is: (180-47) 0.21 = 28 mm Hg which is 34,700 feet. In the experiments, however, minimum survival-time was not reached below 44,000 feet. The tracheal pO<sub>2</sub> at this altitude can be calculated as (116-47) 0.21 = 14.5 mm Hg.

These considerations suggest that the composition of alveolar gases change considerably during and immediately after rapid decompression in a manner that gives a relative advantage in the case of breathing air. Convincing evidence for this theory could only be gained by direct samples of alveolar air taken immediately after explosive decompression.

Procedure. A "parasite compartment" for rapid decompression was designed by H. G. Clamann large enough to accommodate one man in sitting position which was attached to a decompression chamber by a pipe of 12-inch diameter. As the proportion of the two chambers was as 1:40 the smaller compartment could be decompressed to a simulated altitude of 60,000 feet within 2 seconds if the larger one had been previously reduced to 20 mm Hg total pressure. The connection was established by means of a simple knife valve operated by hand on the connecting pipe. The barometric pressure in the chamber was recorded on a photo-kymograph with automatic timing. The subject breathed through a rubber mouthpiece with an expiratory and inspiratory valve which was connected to a gasmeter outside the compartment by means of a reducing valve on the principle of a demand regulator oxygen apparatus. Either air or oxygen could be administered by this device. A nose clamp was used in all cases. Alveolar samples were taken 10 minutes before decompression for base-line values and between 3 and 5 seconds after peak altitude had been reached into evacuated burettes through a tubing which led to the base of the tongue. The burette was opened by the test subject at the end of forced expiration. The oxygen saturation of hemoglobin was recorded continuously at the earlobe with an oximeter designed by Matthes(4). Time of useful consciousness (TUC) was estimated and marked from outside on the general condition and response of the subject to a standard writing test. In one series of

experiments respiratory volumes, electrocardiograms, and electroencephalograms were recorded. Experiments breathing air were started from ground level or 3,000 feet. Using oxygen a base-line at 33,000 feet was maintained for 10-15 minutes before decompression to 50,000 feet and more in order to alleviate disturbances of respiration due to expansion of intestinal gases. To ensure comparable alveolar samples at all altitudes they were all taken by one person. Time of useful consciousness at altitudes of 50,000 and 54,000 feet were checked on Dr. Opitz and showed very little individual variation. No significant impairment due to trapped gas or ear trouble was experienced throughout the entire series of 75 experiments, not even during pressure changes of more than 600 mm in 2 seconds. On rare occasions fits of coughing set in after forced expiration was made to obtain alveolar samples. This irritation persisted for several hours after return to ground level. No lung changes were detected by x-ray examination on the same day.

Table 1. Composition of Alveolar Gas After Decompression Breathing Air

Altitude	% Oxygen	% Carbon Dioxide	% Nitrogen
Ground level	14.3	5.6	79.9
16,400 ft	13.7	8.9	77.3
19,700 ft	14.4	11.1	74.5
21,700 ft	14.8	11.8	73.5
23,000 ft	15.0	10.8	74.2
26,300 ft	17.0	10.8	70.0
29,500 ft	15.4	16.4	68.2
31,200 ft	16.3	17.7	66.0
32,800 ft	19.3	22.3	58.4
33,000 ft	19.8	22.0	58.0
34,500 ft	20.5	24.2	55.8
39,500 ft	23.7	30.7	45.6
40,000 ft	23.4	29.1	47.5
45,900 ft	24.7	41.6	34.4
46,000 ft	26.0	42.0	32.0
50,000 ft	27.0	40.3	32.7



Table 2. Composition of Alveolar Gas After Decompression Breathing Oxygen.

Altitude	% Oxygen	% Carbon Dioxide	% Nitrogen
32,900 ft	71.7	19.5	8.8
32,900 ft	70.6	23.4	6.0
39,400 ft	73.2	22.8	4.0
39,400 ft	72.0	23.6	4.4
42,700 ft	72.8	24.0	3.2 base-level
44,300 ft	66.4	28.0	5.6 3,300 ft
47,600 ft	65.4	31.0	3.6
50,000 ft	58.0	38.8	3.2
50,000 ft	50.6	44.2	5.2
50,000 ft	50.6	45.8	3.6 base-level
52,500 ft	40.0	56.2	3.8 33,000 ft
52,500 ft	41.0	56.0	3.0

Results. In discussing the data presented in tables 1 and 2, it must be kept in mind that each of these findings represents a momentary phase in a rapidly changing course of events in the lungs. Considerable variations even in samples taken at the same altitude in different experiments are to be expected. Taken as a whole, however, each series shows significant trends.

After rapid decompression breathing air (fig. 1), there is:

- a. A steady increase in the CO<sub>2</sub> fraction with altitude rising from 5.6% at ground level to 40% at 50,000 feet. Decompression leads to an initial drop in the partial pressure of CO<sub>2</sub> in the lungs and increases the pressure head through the alveolar membrane.
- b. The oxygen fraction also increases (table 1), but this is not pronounced until altitudes of more than 30,000 feet are reached (fig. 2). At approximately 33,000 feet the alveolar O<sub>2</sub> fraction is similar to that of inspired air. At greater altitudes the oxygen concentration in the alveolar samples is definitely higher than in inspired air. This proves that the tide of oxygen diffusion turns after decompression to great altitudes and may -- at least for a short time -- result in a reflux of oxygen from pulmonary capillary blood into the lungs.
- c. The nitrogen fraction is steadily reduced with altitude so that at 50,000 feet no more than 32% is found in the alveoli. Nitrogen is "flushed out" by carbon dioxide and oxygen.

In the series breathing oxygen (fig. 3) the CO<sub>2</sub> fraction rises as it does breathing air. In contrast the oxygen fraction falls progressively to 40% at 52,500 feet. This does not necessarily imply that no reflux of oxygen takes place in this case. It is probably expelled as fast as it is produced by a greater flush of carbon dioxide. The latter gas has the superiority because of its excellent qualities of diffusion and a greater pressure head from the blood to the lungs.

Gas tensions in the lungs. In calculating the partial pressures of oxygen and carbon dioxide at the time the samples were taken it is

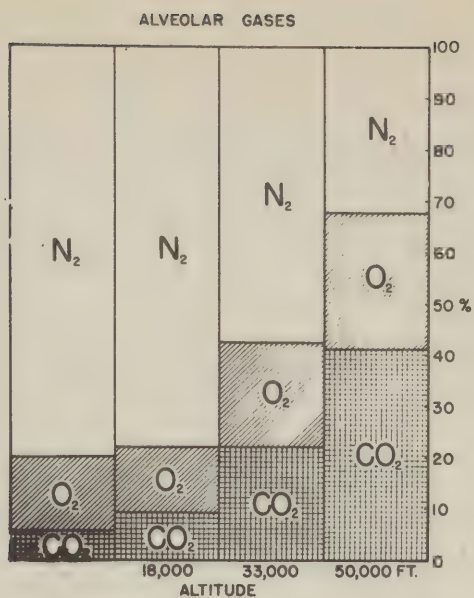


Fig. 1. Composition of alveolar gases after explosive decompression from ground level breathing air.



necessary to make an important reservation: Is it permissible to assume complete water vapor saturation at body temperature under these conditions? During instantaneous decompression 90% of the total gas content of the lungs may be expelled including water vapor. We do not know if complete saturation is regained immediately especially under the following hyperventilation. We hope to find an answer to this question by means of a new device for measuring water vapor on thermoelectric principles being designed by Dr. Clamann. In the meantime we may assume full saturation at the time of sampling. Figure 4 shows alveolar oxygen pressures calculated as  $pO_{2alv} = (B-47) FO_{2alv}$  breathing  $O_2$  and breathing air. The initial advantage gained by breathing oxygen steadily decreases with altitude so that at 50,000 feet it is practically irrelevant if oxygen or air is used. Assuming the oxygen tension in mixed venous blood entering the pulmonary capillaries to be approximately 35 mm Hg even 5 seconds after decompression from ground level, diffusion of oxygen from the blood to the lungs would be possible at altitudes above 29,500 feet breathing air. Using oxygen apparatus this might not occur below 44,000 feet as the venous oxygen pressure would be somewhat higher.

In explosive decompression the lungs play the role of a tonometer for the blood gases and it is evidently possible to shuttle oxygen in one direction or the other by reversing the pressure gradient. From this aspect it is clear why the chances of survival or revival are much better if a rapid descent is effected breathing oxygen. Adequate conditions would be reached at 44,000 feet (fig. 4), whereas, breathing air, not above 22,000 feet.

Instantaneous decompression without oxygen leads to functional impairment more rapidly than the interruption of oxygen supply at the same altitude. In the first case alveolar  $pO_2$  drops to its lowest level simultaneously with ambient pressure. In the latter the oxygen-rich mixture in the lungs is gradually diluted by each breath of air over a period of about 30 seconds, thus increasing the time of useful consciousness. This has been shown at Wright Field recently<sup>(5)</sup>.

Time of useful consciousness breathing 100% oxygen. Explosive decompression to altitudes above 48,000 feet leads to unconsciousness with striking abruptness within 1 minute<sup>(6)</sup>. No preliminary hypoxic symptoms are noticeable. At 50,000 feet the time of useful consciousness is reduced to less than 20 seconds. In more than 20 separate tests between 50,000 and 54,000 feet the TUC was never less than 15 seconds so that we are led to believe that there is a minimum time of useful consciousness which remains constant, similar to the survival time observed in animals.

The samples of alveolar gas had indicated that under these conditions the blood leaving the lungs is very nearly void of oxygen. The oximeter tracings taken from the earlobe (fig. 5) recorded the arrival of partly desaturated blood 4-5 seconds after decompression giving the circulation time from the lungs to the earlobe which we assume to be equal to that of the brain. The oxygen saturation did not reach its lowest level before the 10th second. No impairment of

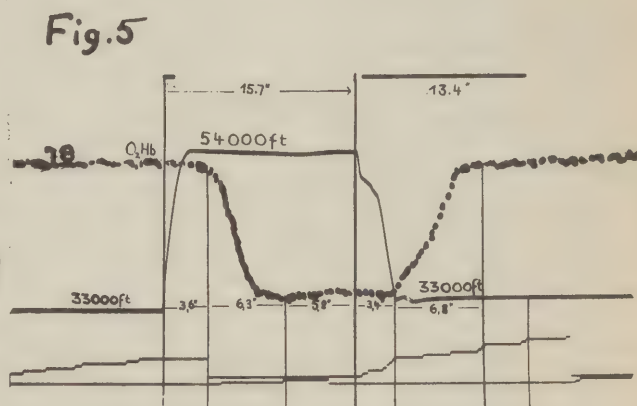
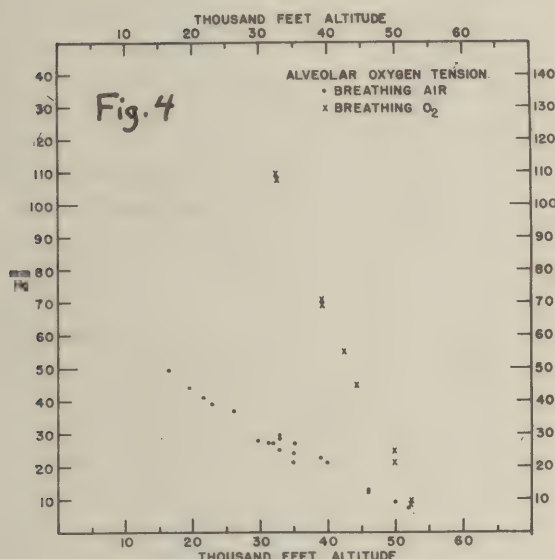
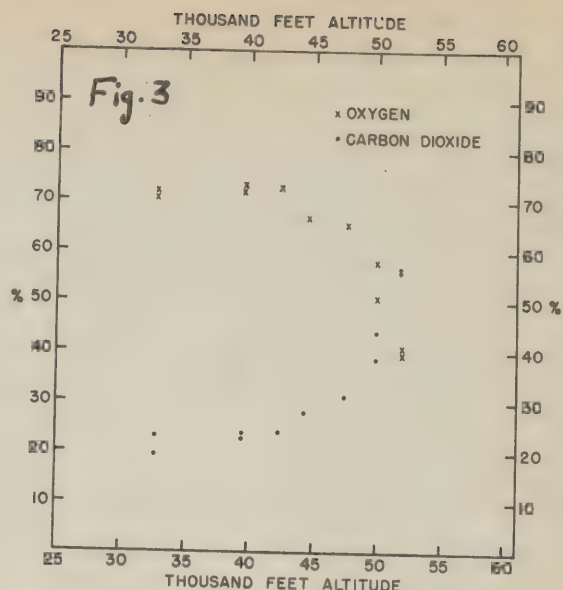
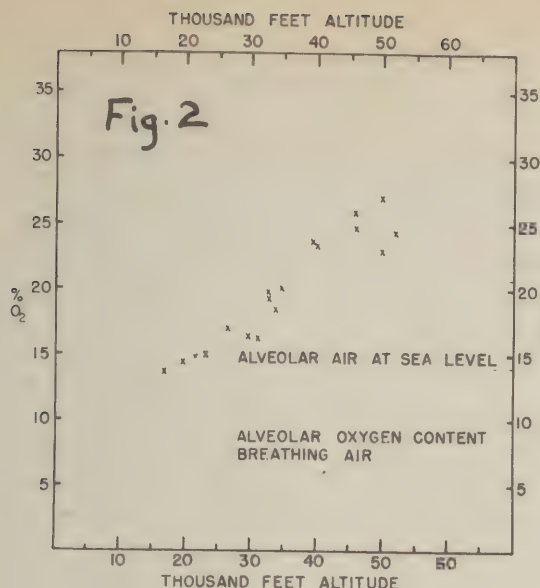


Fig. 2.  $O_2$  content of alveolar samples after explosive decompression breathing air. In comparison: Lower dotted line: mean  $O_2$  content at ground level. Upper dotted line:  $O_2$  content of inspired air.

Fig. 3.  $O_2$  and  $CO_2$  content of alveolar samples after explosive decompression breathing  $O_2$ .

Fig. 4. Alveolar  $O_2$  tension breathing air and breathing  $O_2$  after explosive decompression calculated with  $pH_2O = 47$  mm Hg.

Fig. 5. Recording of barometric pressure (altitude) oximeter tracing at earlobe with signal for loss of consciousness at upper margin.



cerebral function was present in the handwriting test or in the electro-encephalogram until at least 15 seconds had elapsed when unconsciousness set in promptly. The same course of events was reproduced and analyzed in a number of tests on different subjects not varying more than  $\pm 1$  second. The cortical cells of the brain are evidently able to maintain normal function for about 5 seconds after the blood oxygen supply in their vicinity must have reached practically zero, or at least a very low value.

Rapid recompression to 33,000 feet within the TUC invariably led to transitory states of unconsciousness during or after descent independent of altitude after 15-17 seconds provided that the exposure at peak altitude had been more than 5 seconds. Apnea was not observed in any experiments to 52,000 feet ranging to 20 seconds duration. The heart regularly responded with a tachycardia which reached its maximum after 15 seconds.

Summary. 1. During and after instantaneous decompression the composition of the alveolar gas undergoes considerable changes involving an increase of CO<sub>2</sub> diffusion and a diffusion of oxygen from the blood into the alveoli whenever the alveolar oxygen tension drops below that of mixed venous blood. Forced breathing may be beneficial if oxygen has been administered previously and is continued. By hyperventilation the large CO<sub>2</sub> fraction in the lungs can be partly replaced by oxygen. Under the same conditions breathing air hyperventilation is not advisable because it will only release any oxygen remaining in the blood.

2. Above 50,000 feet (with oxygen) a minimum time of useful consciousness of 15-17 seconds remains after rapid decompression.

3. Brief unconsciousness is to be expected even if recompression has been effected before 15 seconds has elapsed independent of altitude.

4. Loss of consciousness could be completely avoided after decompression to 52,000 feet only if recompression had taken place within 5 seconds.

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# RELATIONSHIP BETWEEN AMBIENT AND ALVEOLAR GAS PRESSURES UNDER VARIOUS RESPIRATORY CONDITIONS

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The relationship between alveolar and ambient<sup>1</sup> gas pressures still constitutes a fundamental problem in respiratory physiology. This problem aroused our interest while we were studying the effects of high carbon dioxide pressures on man (1), and the mechanisms involved in pressure breathing (2). These studies led us to speculate on whether or not a simple relationship existed between alveolar carbon dioxide and alveolar oxygen pressures which might hold under all conditions affecting respiration.

Experimental Conditions. Young men were exposed to the following conditions: a, respiration of recirculating air in closed chambers at atmospheric pressure which permitted the accumulation of carbon dioxide to levels as high as 6.75% and depletion of oxygen to levels as low as 10.45% (table 1); b, breathholding maintained as long as possible; c, intermittent pressure breathing using Kreiselman's manual resuscitator at atmospheric pressure to administer gas mixtures containing 9.5, 10.4 and 12% oxygen - respirations were limited to a rate of about 4 per minute and the minute volume to 10 or 11 liters; d, hyperinflation induced by means of a Drinker type respirator<sup>2</sup> with respiration controlled as in c - under both conditions c and d, the same pressure difference (20 cm of water) between alveolar and ambient air was used in ventilating the lungs. The oxygen pressure in the lungs, however, was not increased by more than 2 mm of Hg by the pressure increase per se; e, hyperventilation at a simulated altitude of 15,500 feet.

Results. The relationships between  $-pCO_2$  and  $-pO_2$ <sup>3</sup> are as follows:

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<sup>1</sup>For the purpose of this paper ambient air is considered as air warmed to 37°C and saturated with water vapor.

<sup>2</sup>For purposes of simplicity we have referred to this type of breathing as Respirator type breathing.

<sup>3</sup> $-pO_2$ ,  $-pCO_2$  represents the difference between the ambient and alveolar gas pressure.



Condition a. - breathing recirculated air: at high ambient CO<sub>2</sub> and normal or decreased O<sub>2</sub> in the alveoli, we find an increased pCO<sub>2</sub> (table 2); as -pCO<sub>2</sub> decreases, -pO<sub>2</sub> also decreases.

Condition b. - breathholding: the data (table 3) show that a rise in alveolar pCO<sub>2</sub> is followed by an increased -pCO<sub>2</sub> accompanied by a fall in pO<sub>2</sub> or an increased -pO<sub>2</sub>.

Condition c. - pressure breathing employing normal and decreased oxygen partial pressures: the data (table 4) show that alveolar pCO<sub>2</sub> decreased, -pCO<sub>2</sub> likewise decreased, and -pO<sub>2</sub> showed a corresponding decrease.

Condition d. - hyperinflation utilizing a Drinker type respirator: under this condition (table 4) alveolar pCO<sub>2</sub> decreased, -pCO<sub>2</sub> likewise decreased, and -pO<sub>2</sub> showed a corresponding decrease.

Condition e. - hyperventilation as a result of anoxia at a simulated altitude of 15,500 feet and that induced by inhaling gas mixtures low in oxygen: under this condition alveolar pCO<sub>2</sub> decreased, -pCO<sub>2</sub> likewise decreased, and -pO<sub>2</sub> showed a corresponding decrease (tables 4 and 5).

When the results obtained in all of our tests were plotted, it was observed that a relationship approaching linearity exists between -pCO<sub>2</sub> and -pO<sub>2</sub>.

Table 1. Conditions to which subjects in carbon dioxide experiments were exposed

Exper. No.	No. of men	Duration hours	CO <sub>2</sub> highest attained %	O <sub>2</sub> lowest attained %	Hour when ambient CO <sub>2</sub> reached 5%
1	4	34	5.95	14.18	29
2	4	52	6.54	13.45	34
3	4	51	6.75	19.22	37
4	4	72	5.42	10.45	32
5	37	60	5.27	12.21	34
6	77	50	5.18	13.21	34

Table 2. Effects of high ambient carbon dioxide on alveolar air composition

Date	No. of Subjects	Hr. of exposure	Ambient air mm. Hg		Alveolar air mm. Hg		- pCO <sub>2</sub>	- pO <sub>2</sub>
			pCO <sub>2</sub>	pO <sub>2</sub>	pCO <sub>2</sub>	pO <sub>2</sub>		
1944								
5/11	4	Rest	0.2	150.1	40.8	100.4	40.6	49.7
		4	9.2	140.5	39.3	108.7	30.1	36.8
		10	17.3	131.2	42.3	98.5	25.0	32.7
5/12		23	27.5	119.1	43.5	95.0	16.0	22.1
		28	34.3	111.0	46.4	92.5	12.1	18.5
		34	42.6	101.5	50.4	89.3	7.8	12.2
5/17	4	Rest	0.2	150.1	41.9	103.0	41.7	47.0
		4	5.3	143.4	33.7	112.8	26.6	30.6
		10	12.6	134.4	40.4	100.0	27.8	34.4
5/18		22	22.3	123.7	42.2	95.2	19.9	28.5
		28	28.8	116.3	44.1	92.9	15.3	23.4
		34	34.1	109.6	46.3	89.0	12.2	20.0
5/19		46	40.0	102.8	49.3	88.2	12.3	18.4
		51	46.2	95.2	55.8	81.4	9.6	13.8
5/25	4	Rest	0.2	150.1	41.1	98.1	39.9	52.0
		18	15.9	136.5	41.4	105.6	25.5	32.9
		34	31.0	147.5	46.8	127.0	15.8	20.5
5/26		42	38.8	140.0	50.7	123.7	11.9	16.3
		51	48.2	147.2	56.7	135.8	8.5	11.4
5/31	4	Rest	0.2	148.5	35.5	108.2	35.3	40.3
6/1		17.5	17.4	127.5	35.0	102.2	20.6	25.3
		28	29.4	114.4	42.9	95.0	13.4	19.4
6/2		42	32.3	106.8	46.0	91.2	13.5	15.6
		52	35.0	93.2	46.5	76.1	11.5	17.1
		58	33.6	87.3	45.9	73.5	12.3	13.8
6/3		66	30.6	92.6	44.4	70.5	13.8	22.1
		72	36.2	73.5	44.5	60.7	8.3	12.8
7/13	10	Rest	0.2	148.8	42.3	97.6	42.0	51.2
7/14	8	19	22.2	122.7	45.5	90.6	22.3	32.1
7/15	10	31	30.7	110.0	49.6	84.4	18.9	28.6
7/16	7	54	35.2	90.8	48.5	72.1	13.3	18.7



Table 3. Effects of breath holding on alveolar air composition

Subject	Alveolar air mm. Hg		- pCO <sub>2</sub>	- pO <sub>2</sub>
	pCO <sub>2</sub>	pO <sub>2</sub>		
CON	44.8	82.5	44.5	66.5
	43.3	90.3	43.2	58.7
	44.4	81.8	44.2	67.8
MOO	42.3	97.3	42.1	51.7
	39.5	95.3	39.3	53.7
	45.1	80.7	44.9	68.3
MAR	45.7	91.0	45.5	58.0
	46.1	93.7	45.9	55.3
CEC	41.6	91.4	41.4	57.6
	49.8	71.0	49.6	78.0
	47.2	81.0	47.0	68.0
ONE	46.5	85.9	46.3	63.1
	44.9	88.3	44.7	60.7
	45.6	84.5	45.4	64.5
SAR	50.3	82.8	50.1	66.2
	51.0	74.7	50.8	74.3
	52.6	74.7	52.4	74.3

Table 4. Effects of positive and negative pressure breathing on alveolar air composition

Sub- ject	Date	Time minutes	Alveolar air mm. Hg		- pCO <sub>2</sub>	- pO <sub>2</sub>	
			pCO <sub>2</sub>	pO <sub>2</sub>			
NP	1943 1/22	0-5	40.5	79.8	40.3	69.5	Room air -voluntary breathing
		6-10	29.3	124.1	29.1	25.2	" " -Respirator
		16-24	38.5	38.3	38.5	47.5	12.0 per cent O <sub>2</sub> - voluntary breathing
		25-43	28.3	59.6	28.3	26.2	12.0 per cent O <sub>2</sub> -Respirator
	-	35-39	32.1	33.9	32.1	51.8	" " " -vol.breathing
BV	1/23	0-5	38.9	93.8	38.7	54.3	Room air-voluntary breathing
		6-10	32.6	117.3	32.4	30.8	" " -Respirator
		11-15	35.4	92.9	35.2	55.2	" " -voluntary breathing
		16-25	36.1	44.5	36.1	40.6	12.0 per cent O <sub>2</sub> vol. "
		26-35	29.5	55.0	29.5	30.1	" " " " -respirator
		36-45	31.4	45.7	31.4	39.4	" " " " -vol.breathing
		46-55	27.3	56.5	27.3	28.6	" " " " -respirator
		56-60	30.5	40.9	30.5	44.2	" " " " -vol.breathing
		61-69	32.0	96.0	31.8	52.1	Room air-voluntary breathing
VB	1/24	0-10	33.1	39.4	33.1	33.9	10.4 per cent O <sub>2</sub> -vol.breathing
		11-20	25.9	51.6	25.9	23.3	10.4 " " -pressure breathing
		21-25	27.0	39.2	27.0	34.1	10.4 per cent O <sub>2</sub> -vol.breathing
		26-35	24.4	44.9	24.4	28.1	10.4 " " " -respirator
		36-40	28.5	33.9	28.5	39.1	10.4 " " " -vol.breathing
ARB	1/23	0-5	41.5	88.0	41.3	60.1	Room air-voluntary breathing
		6-10	35.1	111.5	34.9	36.6	" " -respirator
		11-15	46.4	82.3	46.1	65.8	" " -voluntary breathing
		16-25	35.5	37.1	35.5	30.2	9.5 per cent O <sub>2</sub> -vol.breathing
		26-35	31.7	34.2	31.7	33.1	" " " " -respirator
		36-45	29.0	36.2	29.0	31.1	" " " " -vol.breathing
		46-55	28.5	35.2	28.5	32.1	" " " " -respirator
		56-58	27.9	33.8	27.9	33.5	" " " " -vol.breathing
NP	1/23	0-12	25.7	68.1	25.7	17.0	12.0 per cent O <sub>2</sub> -pressure
		0-12	23.5	67.0	23.5	18.1	breathing



Table 5. Effects of altitude on alveolar air composition

Day of exp.	No. of subjects	Alveolar air in mm. Hg		= pCO <sub>2</sub>	= pO <sub>2</sub>
		pCO <sub>2</sub>	pO <sub>2</sub>		
<u>Ground Level</u>					
1	4	40.5	103.5	40.3	46.0
3	4	39.8	101.1	39.6	47.4
18	4	40.9	103.0	40.7	46.5
25	4	39.9	99.7	39.7	49.8
32	4	41.4	97.6	41.2	52.8
39	4	41.5	95.8	41.3	53.7
46	4	41.6	96.2	41.4	53.3
53	4	41.5	99.1	41.3	50.4
60	4	41.2	98.0	41.0	51.5
64	4	89.6	98.3	39.4	50.2
Av.		40.8	99.2	40.6	50.2
<u>15,500 Feet in Altitude</u>					
4	4	32.9	37.6	32.7	36.6
7	4	33.5	42.2	33.3	31.0
21	4	35.0	39.8	34.8	34.4
28	4	35.2	39.8	35.0	34.4
35	4	34.4	39.3	34.2	34.9
42	4	35.0	39.0	34.8	34.2
49	4	35.4	37.9	35.2	35.3
56	4	34.0	40.8	33.8	32.4
Av.		34.4	39.6	34.2	34.2

Comments. It should be noted that the plot terminates at values of approximately 10 mm.  $\sim$  pCO<sub>2</sub> and pO<sub>2</sub>. The limits imposed by the mechanical limitation of the respiratory system have been reached and no more ambient air can be brought into the lungs by means of this mechanism to compensate for the rate of excretion of carbon dioxide and the rate of uptake of oxygen.

Similarly, there is a limit at the other extreme. In this case  $\sim$  pO<sub>2</sub> is limited to a value of the order of 80 mm., and pCO<sub>2</sub> in the alveoli will rarely increase above 50 mm. Between these two extremes one may draw a smooth curve. There is considerable scattering of points but despite the fact that these data were obtained using different groups of subjects under various ambient oxygen, carbon dioxide, and breathing conditions, it is apparent that a specific correlation has been demonstrated between  $\sim$  pCO<sub>2</sub> and  $\sim$  pO<sub>2</sub>.

The significance of this correlation is that  $\sim$  pCO<sub>2</sub> is in all probability a function of the degree of effective ventilation.

As  $\sim$  pO<sub>2</sub> decreases, pulmonary ventilation may be said to become more effective. Thus  $\sim$  pO<sub>2</sub> may find significance as an index of effectiveness of pulmonary ventilation.

Last year Gray presented a mathematical formulation for respiratory ventilation (3) and Fenn did the same for respiration under various anoxic conditions (4). The simplicity of the relation of  $\sim$  pCO<sub>2</sub> to  $\sim$  pO<sub>2</sub> may lend itself to applications in respiratory physiology.

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# THE RESPIRATORY EXCHANGE IN DROSOPHILA DURING FLIGHT

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Among alternating movements in animals, the wing motion of certain insects is unique in its high frequency. Rates of 200 or more wing beats per second are common. These figures may be contrasted with known rates of alternating movement among vertebrate animals, which are approximately: 100 per second for the rattling of *Crotalus* at 40°C; 75 per second for the wing motion of the humming bird; 20 per second for the reflex scratching of a mouse; and 10 per second for various alternating movements in man. With *Drosophila* in laboratory experiments, flights lasting upwards of two hours have been observed stroboscopically and it has been calculated that during this time over a million successive contractions of the opposing groups of flight muscles will have occurred.

Information as to the metabolic processes concerned in the intense activity characteristic of the flight of insects has been sought in continuous measurements of the rate of wing beat and of the respiratory quotient. In *Drosophila americana* during flight, the respiratory exchange, as determined by the technique of Fenn (1928) averages 13 times the previous resting level and varies in proportion to the third power of the rate of wing beat. At the outset of flight, when the rate is at a maximum, oxygen uptake or carbon dioxide output may reach levels as high as 25 times the previous resting rate. Both frequency of wing beat and the respiratory exchange decline in the later stages of prolonged flights, but the respiratory quotient remains at a value of 1.

This indication that carbohydrate is the principal or possibly the only source of flight energy in these flies is in quantitative agreement with determinations made by other workers of the rate of disappearance of glycogen during flight. Post-flight respiratory quotients are often as low as 0.7 or less, and suggest a nearly complete exhaustion of the carbohydrate reserves. although survival may be prolonged for as much as 24 hours.

The observation that respiratory rate is proportional to the cube of wing frequency is in accord with theoretical expectations derived from a comparison of the wing movement with simple harmonic motion and from a study in which the relationship between wing frequency and bodily proportions was measured for some 25 species and races of *Drosophila*. Observed variations from the expected proportionality were of a random nature and are attributed to imperfect experimental technique and to inability to measure small changes in the amplitude and angle of attack.

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## THE RESPIRATORY SYSTEM OF THE PIGEON

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The pigeon has been found to differ remarkably from mammals in its reaction to various inhaled compounds. A review of the literature reveals that little work on the respiratory system along anatomical lines has been done on the pigeon per se, but the author has found previous fowl studies (1-8) are applicable to the pigeon.

The upper respiratory system consists of a nasal cavity which is short and narrow and divided by a septum composed of bone and cartilage (1,2). Three mucous-membrane-covered plates project into each half of the nasal cavity which are comparable to the mammalian turbinates (2); however they are not nearly as extensive. The nasal cavity is connected to the pharyngeal cavity through the palatine cleft (2).

The Larynx is not guarded by an epiglottis as in the mammal (7) but is merely an elliptical slit at the base of the tongue. The cranial larynx, as it is called, is devoid of thoroid cartilages and vocal cords. The cricoid cartilage is segmented into one dorsal, two lateral, and one ventral portion (3). The arytenoid cartilage is elongated.

A relatively long trachea composed of complete rings of cartilage connects the cranial larynx with the caudal larynx or syrinx (3), the true organ of voice of the pigeon. The syrinx is indicated by a lateral compression where the trachea divides into the two bronchi (3). On the medial internal surface are two elastic membranes which serve as vocal cords (8). These may be compared with the vocal folds of the mammalian larynx. The lungs, which are small in comparison with the size of the thorax, are closely applied by their dorsal surface to the vertebral column and the ribs. There is no actual pleural space. Since they are firmly attached they are comparatively nonexpansile. The ventral surface is free and covered with pleura and the rudimentary tendinous diaphragm. The pigeon does not have a muscular diaphragm, as have mammals, dividing the thoracic and abdominal cavities.

The ventral surface of each lung is pierced by a bronchus, which passes through the whole length of the lung and communicates finally with the greater and lesser abdominal air sacs. Within the lung the main bronchus gives off secondary bronchi which communicate directly or indirectly with the remaining air sacs. There are 9 air sacs in the pigeon. The first and most anterior are the paired thoracocervical sacs. Prolongations of these extend anteriorly through the



canalis transversarius of the cervical vertebrae as far forward as the fourth. Posterior extensions of the sacs may extend back as far as the fourth thoracic vertebrae. A large lateral extension of each sac extends between the scapula and the thoracic wall.

The anterior thoracic air sac, as its name indicates, is located in the anterior part of the thoracic cavity. This is a single sac which has pneumatic connections with the pneumatic cavities of the bones of the shoulder girdle, humerus, sternal ribs and sternum. The posterior thoracic air sacs are paired and occupy a space ventral to the lungs. The lesser abdominal air sacs are paired. These are laterally compressed triangular sacs located in the anterior part of the abdominal wall about its middle third. The greater abdominal air sacs are also paired (2). These sacs are distributed throughout the abdominal cavity and surround a large portion of the viscera. Pneumatic connections are made to the pelvis and sacral vertebrae.

In order to demonstrate and determine the extent of the air sacs and lung structure it was necessary to make some type of permanent replica of the respiratory system. This was done by injecting plastic into a dead pigeon and dissecting the pigeon away after the plastic had hardened.

Mechanism of Respirations. The mechanism of respiration in the pigeon is markedly different from that of the mammal (5,6). On inspiration the air passes down the trachea through the primary and secondary bronchi directly into the air sacs without passing through the fine capillary tubules. Therefore little gaseous exchange takes place during inspiration as can be demonstrated by removing a sample of air from one of the air sacs and analyzing it. The air removed from the air sac closely approximates that of atmospheric air. On expiration the air passes through the capillary structure of the lung by a different passage during which gaseous exchange takes place.

Since the lungs are so firmly attached to the dorsal surface of the thorax, and no muscular diaphragm is present, they expand very little if any during respiration. The air sacs act as reservoirs for the inspired air. The muscular power behind respiration is primarily abdominal and alternation in intra-abdominal pressure alternately inflates and deflates the air sacs.

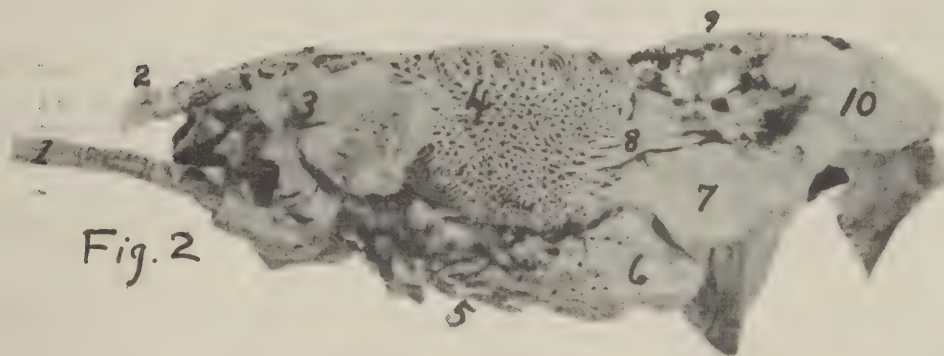
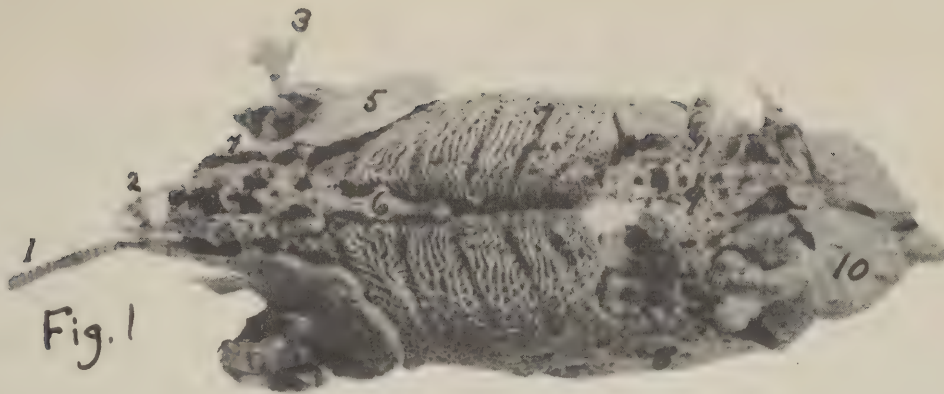


Fig. 1,2. Plastic Cast, Respiratory System of Pigeon. Fig. 1, Dorsal View. Fig. 2, Lateral View.

1--Trachea. 2--Pneumatic cervical extension. 3--Thoraco-cervical air sac. 4--Capillary lung structure. 5--Pneumatic cavity of sternum. 6--Posterior thoracic air sac. 7--Lesser abdominal air sac. 8--Recurrent bronchi. 9--Pneumatic cavity of sacral vertebrae. 10--Greater abdominal air sac.



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# STUDIES ON RESPIRED WATER VAPOR

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Two years ago we reported (1) the results of a few determinations of the exhaled water vapor of human subjects breathing dry oxygen at ground level and 30,000 feet simulated altitude. At this time are presented additional data obtained at altitude, as well as experiments showing the effects of density and respiratory rate changes.

Exhaled gas was passed by means of a rubber nosepiece and warmed metal check valve into either a chilled brass canister or chilled copper coil, where the water vapor was frozen out and weighed (2). The gas then passed into a recording spirometer, where it was mixed and where the temperature and degree of saturation were determined by means of wet and dry thermocouples. Inhalation was through a plastic valve, the oxygen being supplied by a Pioneer diluter demand regulator.

The canisters and coils were weighed to a precision of 5 mgm. in 1500, or 0.3%. Calibration of the system with known amounts of water showed a mean recovery of -2.69%,  $\pm$  1.529.

Subjects were young enlisted men made available to us by the Navy, and except for an indoctrination flight, were inexperienced in altitude chamber work. Each had been given a recent physical examination, and was free of respiratory infection at the time of the experiment.

The analysis of variance of the early data had shown the necessity of pairing the experiments, in order to avoid the confounding effect of the variation between subjects as well as within subjects on different days. The ground level tests were made after the subject had been at rest and on full oxygen for at least 15 minutes. Ascent at 3,000 feet per minute was started immediately upon completion of the ground test.

TABLE 1. Respiratory Data and Vapor Pressures of 12  
Subjects at Rest Breathing Oxygen.

	Respir. PerMin.	Min. Vol. L.STPaD	Mgm H <sup>2</sup> O Per Min.	Vap. Pr. From STPaD <sup>1</sup> Vols.	Vap Pr. From Ex- haled <sup>2</sup> Vols.	Vap Pr. From Dew Points
				Mm. Hg	Mm. Hg	Mm. Hg
Ground	13.8	7.9	244	33.8	28.5	29.9
Altitude	13.5	5.8	195	35.6	27.4	28.3
	-0.3	-2.1	-49	1.86* $\pm$ 1.92	-1.1**	-1.6**
%	-2	-27	-20	$\pm$ 6	-4	-5

1. Volumes corrected to standard conditions: 0°C., 760 or 226 mm. Hg pressure, dry.

2. Volumes corrected to exhaled conditions: 32.2°C., 760 or 226 mm. Hg pressure, 80% saturated.

\* and \*\* indicate significance at the 5% and 1% levels respectively.



Direct measurements of the dewpoint of the exhaled air were made by passing the air over the polished surface of a massive copper bar, along which a temperature gradient was maintained. The point at which condensation began was clearly visible, and its temperature was determined by means of thermocouples placed just below the bar surface. This dewpoint method has the advantage of speed and of being independent of calculations based on respired volumes.

Exhaled gas temperatures were obtained by a thermocouple placed close to the nostrils. The measured temperatures were not correlated with rate of respiration, so we feel confident that we were accurately approaching the maximum exhaled temperatures.

The effect of density of the inspired gas on vapor pressure was studied by having the subjects breathe a mixture of 23% oxygen in helium. The density of this mixture is approximately that of 100% oxygen at 30,000 feet. This mixture was breathed at the subjects' own rate, and also at a predetermined imposed rate.

TABLE 2. Effect of Change in Density of Respired Gas on Respiratory Rate and Aqueous Vapor Pressure.

Respired Gas	No. of Obser.	Respir. Per Min.	Vapor Pr. From STPaD Vols.		Vapor Pr. From Dew Points	
			Mm. Hg	—	Mm. Hg	—
O <sub>2</sub>	10	11.9	36.7			
He + O <sub>2</sub>	10	13.0	35.9	-0.8 (P=1-.2)		
O <sub>2</sub>	105				31.9	
He + O <sub>2</sub>	105				32.2	+0.24*
O <sub>2</sub>	8	13.2	36.7			
He + O <sub>2</sub>	8	13.1	35.5	-1.2*		

\* Indicates significance at the 5% level.

Vapor pressures were calculated by dividing the weight of water collected by the liters of respired gas corrected to either standard volume or to the exhaled volume, which meant to 32.2°C, ambient pressure, and 80% saturated. It seems difficult to justify use of this latter correction, where a constant value must be assumed for the same quantity whose change is being investigated.

Results of the altitude tests are shown in table 1. At altitude, 9 of 12 subjects increased the vapor pressure calculated from standard

volumes; analysis of variance indicated that the mean increase of approximately 2 mm. Hg. for the whole group was significant. Vapor pressures calculated from exhaled volumes were consistently lower at altitude, which is corroborated by the dewpoint measurements made on 8 of the 12 subjects. The reversal of sign of the ground-altitude vapor pressure differences indicates the critical nature of the calculations.

Table 2 shows the effect of density changes upon the vapor pressures. When the subjects breathed at their own respiratory rates, there was no significant mean difference in the vapor pressures. There was also a marked slowing of the respiratory rate. When they breathed the helium mixture at the same rate as they had the oxygen, the vapor pressures were lowered significantly. This would indicate that the effect of the less dense gas was to both slow the respirations and lower the vapor pressure. Since it is well-known (3) that slowing the respiration raises the vapor pressure, this would tend to cancel out the vapor pressure drop due to the density change, and would explain why no significant change in vapor pressure was found when the rate was unregulated.

TABLE 3. Correlation of Aqueous Vapor Pressure (Based on STPaD Vols.) with Exhaled Gas Temperature and Respiratory Rate.

Condition	No. of Obser.	Respir. Per Min.	Temp. °C.	Vapor Pr. Mm. Hg	r
Ground	24	13.1	32.0		-.2596
"	24		32.0	36.6	+.7836**
Ground	15	13.7		32.5	
Altitude	15	13.6		34.0	
--		-0.1		+1.5*	-.4984*
O <sub>2</sub>	26	12.8		36.8	
He + O <sub>2</sub>	26	13.1		35.8	
--		+0.3		-1.0	-.2584
O	8	13.2		36.7	
He + O <sub>2</sub>	8	13.1		35.5	
--		-0.1		-1.2*	-.3929

\* and \*\* indicate significance at the 5% and 1% levels respectively.

The temperatures of the exhaled gas and the vapor pressures were highly significantly correlated (table 3) when the subjects were at rest breathing oxygen at ground level. Significant temperature changes did not accompany the vapor pressure changes due to breathing the helium mixture at the same rate as the oxygen. We do not have data on



exhaled gas temperatures at altitude.

The relation of aqueous vapor pressure and respiratory rate was fairly evident. There was a significant inverse correlation ( $r = -.4984^*$ ), between the rate changes and changes in vapor pressures calculated from standard volumes of the 15 subjects tested at ground level and altitude. On the helium mixture the correlation between rates and vapor pressures of all subjects tested was small. It approached significance when we used the data from only those subjects whose rates were unchanged.

We have not done the crucial experiment of attempting to reproduce the effect of going to altitude by having the subjects breathe at their altitude rate. Probably it would also be necessary to substitute the less dense gas mixture in order to duplicate on the ground the altitude results.

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# SURVIVAL TIME OF ANIMALS EXPOSED TO EXTREMES OF ALTITUDE

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Explosive decompression per se up to 75,000 feet does not kill animals. We have found this to be the case in unanesthetized monkeys as well as rats when they are recompressed at free-fall rate immediately after the explosive decompression. The half time of decompression was 0.04 seconds. The rate of recompression was 20,000 feet per minute down to 30,000 feet altitude and from there to sea level at the rate of 12,000 feet per minute. Continuous recording of respiration, EKG, blood pressure and EEG was maintained throughout the experiment.

As in rats, respiration in monkeys ceases shortly after decompression. The respiratory center, however, spontaneously recovers when the animals are recompressed. This occurs both in oxygen and in air.

The irregularity in the heart rate noted immediately after decompression is invariably followed by marked bradycardia. The latter is also reversible, as in the case of respiration, and the records strongly indicate that the bradycardia is at least in part the result of the complete anoxia resulting from decompression at high altitude and the temporary arrest of respiration. Bilaterally vagotomized monkeys have been found unsuitable for explosive decompression experiments and the role of the vagus has not as yet been determined. Simultaneous blood pressure recordings show a marked reduction, particularly in diastolic pressure, following decompression. The records indicate that the course followed by respiration, heart rate, and blood pressure following decompression parallel each other to a considerable degree.

Of the few monkeys so far tried, it has been found that the rate of survival when recompressed at free-fall rate after explosive decompression is about the same as that found in rats. The monkeys seem to be as hardy and have survived decompression at 75,000 feet in oxygen and at 57,000 feet in air.



The survival of rats descending at free-fall rate from high altitude after explosive decompression is given in the following table:

Decompression Altitude in Feet						
	75,000	70,000	65,000	60,000	55,000	50,000
Percentage Survival						
In air			0	25	37.5	100
In oxygen	50	75	85	100		

Since the barometric pressure at 52,500 feet is about equal to the sum of the alveolar vapor and carbondioxide pressures, animals descending from simulated high altitudes in oxygen or in air continue to be under complete anoxic conditions until the 52,500-foot level is passed. When recompressed in oxygen, a tolerable level is reached between 40,000 and 45,000 feet. When recompressed in air, however, it is not until about 20,000-foot altitude is reached that a tolerable level is attained. At these two altitudes, one in pure oxygen and the other in air, the arterial oxygen saturation should be about 65%. An animal descending at free-fall rate from a decompression altitude of 50,000 feet in air will require 110 seconds to reach the tolerable 20,000-foot level. In this case, from the moment descent begins, some oxygen is available and it becomes progressively more significant, and as the above table indicates, 100% of such animals survive. While it requires only 99 seconds to reach the 42,000-foot level when descending in pure oxygen from 75,000 feet, most of the time in this descent is in complete anoxia, and only 50% of such animals survive.

## PHYSIOLOGICAL EFFECTS OF EXPLOSIVE DECOMPRESSION

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Investigations of the physiological effects of explosive decompression on both men and animals have been under way in this laboratory since 1942. These investigations as well as similar work carried on at the Aero-Medical Laboratory at Wright Field and elsewhere have shown that explosive decompression to altitudes as high as 45,000 feet in less than one-tenth of a second do not result in a serious hazard to fliers. In the laboratory at Ohio State University we have demonstrated that explosive decompression produced a slight increase in susceptibility to the 'bends'. In work on animals we have shown that there is a slowing of the heart and a drop in blood pressure at the time of the explosion. Recovery from the drop in blood pressure occurs within one minute. There is also a very brief elevation in the intrathoracic pressure which results from the fact that the drop in ambient pressure occurs more rapidly than does the drop of pressure in the lungs, since the maximum rate of decompression of the lung is determined by the cross-section area of the trachea and the rate at which air can escape through this tube.

Recent experiments have been carried out on the physiological effects of explosive decompression to altitudes of 70,000 feet and higher. Under these conditions the animals (both dogs and albino rats have been used) collapse and exhibit anoxic convulsions within ten seconds after the explosion. A few seconds later, subcutaneous swelling begins. This usually starts in the hind quarters and progresses forward. The swelling occurs suddenly as though the subcutaneous fascia were being torn and the skin separated from the underlying tissues. Upon recompression the swelling disappears almost instantly when the pressure reaches approximately 50 mm. Hg. Animals recover within one-half hour after being brought back to ground level provided the stay at the pressure of 30 mm. or less was not too long (90 seconds for dogs and about one minute for rats). This subcutaneous swelling is believed to be the result of the vaporization of tissue fluids.

A study of the rapidity with which the alveolar air comes into equilibrium with the blood following a rapid change in barometric pressure has been under way for some months. To make possible a satisfactory study of the changes in composition of alveolar air that occur following such rapidly induced changes in ambient pressure it was necessary to develop an analytical instrument that would record accurately rapid changes in composition of the air leaving the lungs. We have, therefore, designed and constructed a mass spectrometer of the Neir type, specially adapted for the analysis of respiratory gases. The instrument makes possible the simultaneous analysis for oxygen, nitrogen, and carbon dioxide. A special calibrating arrangement permits the quantitation of each of these components. The instrument records



the concentrations of all three components on a single photographic film. The ion source is so constructed that analysis is continuous. The time required for recording full scale concentration change is less than two-tenths of a second. The analytical error of the instrument is less than 2% of full scale in normal use. An expanded scale provision has been incorporated which magnifies the changes, thus increasing the accuracy. Preliminary analyses of alveolar air expulsions have not verified the existence of an oxygen trough. This problem is being studied further.

This work was done under contract with the Office of Naval Research. This is a cooperative project which has been carried out by the Department of Physiology with the help of the Department of Electrical Engineering and the Department of Physics. Personnel involved are as follows: from the Department of Electrical Engineering, Dr. Theodore J. Wang; from the Department of Physics, Mr. Jack A. Hunter, and the the Department of Physiology, Dr. W. V. Whitehorn, Dr. Abraham Edelmann, Dr. Floyd Beman, Mr. Ralph W. Stacy and Dr. F. A. Hitchcock.

SOME FACTORS WHICH DETERMINE THE FLOW OF OXYGEN  
FROM ALVEOLAR AIR TO ARTERIAL BLOOD

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The errors inherent in the usual methods of sampling alveolar air at rest become large when the subject exercises: the samples contain an excess of CO<sub>2</sub> and a deficit of O<sub>2</sub>. To avoid these errors an indirect method has been devised for determining the physiologically effective pressures of these respiratory gases. The method depends on the interrelations of pCO<sub>2</sub> and pO<sub>2</sub> in inspired and expired air (R.Q.) and the virtual identity of alveolar and arterial pCO<sub>2</sub>. From determinations of the pCO<sub>2</sub> of arterial blood and expired air, and of the pO<sub>2</sub> of inspired and expired air, the mean alveolar pO<sub>2</sub> may be calculated.

By means of this method the oxygen pressure gradient between alveolar air and peripheral arterial blood (A-A) has been measured in man during rest and exercise at sea level and during anoxia. At rest the A-A gradient averaged 9 mm. Hg; during exercise (O<sub>2</sub> consumption = 1.5 l./min.) the A-A gradient averaged 16 mm. Hg. These A-A gradients were the same during states of normal and reduced oxygenation.

The A-A gradient results from two components: an admixture of venous blood to arterialised blood (e.g. metabolism of lung) and the "membrane" barrier interposed by tissue and fluid between the alveolus and intracellular hemoglobin. By means of certain assumptions, it can be calculated that at sea level the A-A gradient is formed almost completely by the venous admixture component; at altitude, however, the A-A gradient is owing almost entirely to the membrane component. Exercise, by exerting stress on the mechanisms serving the transfer of oxygen from alveolar air to arterial blood evokes an integrated series of respiratory and cardiovascular adaptations, one of which is an increase in the A-A gradient.

A detailed report of this study has been published: Amer. Jour. Physiol., 147, 191-198, 199-216, (1946).





# THE PHENOMENON OF DIFFUSION RESPIRATION<sup>1</sup>

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By diffusion respiration is meant gas-exchange between the atmosphere and the lung alveoli (more particularly oxygen intake) in the absence of respiratory movements of the chest and without the intervention of any sort of artificial respiration. It is a form of natural respiration, the energy for which is supplied by the body. Under certain conditions to be discussed later, diffusion respiration is capable of providing enough oxygen to keep dogs alive for periods up to an hour and a half after respiration has ceased. The phenomenon has been elaborated in several papers from this laboratory.

## "HEMOGLOBIN-OXYGEN PUMP"

A schematic diagram of this pump is presented in figure 1. The manner of its action is as follows. As long as blood is circulating through the lungs a constant stream of reduced hemoglobin passes through the respiratory capillaries. The oxygenation of this reduced hemoglobin removes oxygen from the respiratory spaces and consequently lowers the total barometric pressure within this area slightly below that of the atmosphere at the glottis. A diffusion inwards of the dead space air and the atmosphere en masse results.

The "hemoglobin-oxygen pump", therefore, is an inspiratory mechanism, the effect of which is to apply a continuous vis a fronte upon the dead space air and the atmosphere at the glottis. The ability of this pump to postpone asphyxial death on cessation of the respiratory movements depends upon the conditions present when the arrest occurs.

Respiratory Arrest in Air. Under this circumstance the gas sucked inwards to the alveoli by the pump is rich in nitrogen and poor in oxygen. The oxygen of this gas is rapidly and selectively absorbed into the blood stream, leaving behind the nitrogen. The nitrogen, however, is neither removed by the blood nor evacuated by expiratory movements and consequently it accumulates at the expense of the alveolar space available for oxygen. The outcome of this series of events is that nitrogen, within a matter of minutes, occupies such a large proportion

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<sup>1</sup>These investigations have received financial support under Naval Task Order #1, N6-ori-131.



of the alveolar space that the oxygen pump becomes unable to maintain the alveolar tension of oxygen required to sustain life and asphyxial death ensues.

The implication of the above is that the higher animals have two mechanisms for the inspiration of gas but only one for its expiration. Respiratory arrest, therefore, although it involves a practically complete cessation of the periodic evacuation of nitrogen, leaves in functional operation, for a period of time, a mechanism capable of producing the inspiration of oxygen. The "hemoglobin-oxygen pump", however, sucks in large amounts of nitrogen with oxygen, with the result that the mechanism is thereby quickly rendered impotent and the animal dies, suffocated by the accumulating, unexpired nitrogen.

Respiratory Arrest Under Conditions Favorable to Diffusion Respiration. For successful diffusion respiration the following conditions must be present:

a. The nitrogen of the atmosphere at the glottis, the dead space air and the blood and tissues, etc., must have been largely replaced by oxygen.

b. The airway must be patent. An obstructed airway leads to the development of a negative intrapulmonary pressure and to collapse of the lung.

c. The circulation must be adequate. The power of the "hemoglobin-oxygen pump" to exert an inward suction is proportional to the rate of the flow of reduced hemoglobin through the respiratory capillaries.

#### Experimental Procedure

The following procedure applies to most of our experiments. Mongrel dogs were used and the experiments were carried out under thio-pental (pentothal) sodium anesthesia. After induction of second-plane anesthesia the necessary surgical procedures were carried out and a partial denitrogenation was accomplished by forcing the animal to breathe pure oxygen for 45 minutes. After completion of the process of denitrogenation, respiratory arrest was induced and maintained for a standard period of 45 minutes by the infusion of an overdose of pentothal sodium. In order to prevent the reappearance of spontaneous respiration it was found necessary to administer an average of 10.0 mg. of pentothal sodium per kilogram during the first 30 minutes of arrest. By this time the animal had passed into an autogenous carbon dioxide anesthesia and no further anesthetic was required. In some experiments the dog was resuscitated by the intratracheal insufflation of oxygen; in others the animal was sacrificed.

Demonstration of "Hemoglobin-Oxygen Pump". In one series of 15 experiments, the animal, after denitrogenation, was attached by a tracheal cannula to a sensitive balanced spirometer equipped with a soda lime chamber. In figure 3 it can be seen that the uptake of oxygen

# THE HEMOGLOBIN-OXYGEN PUMP

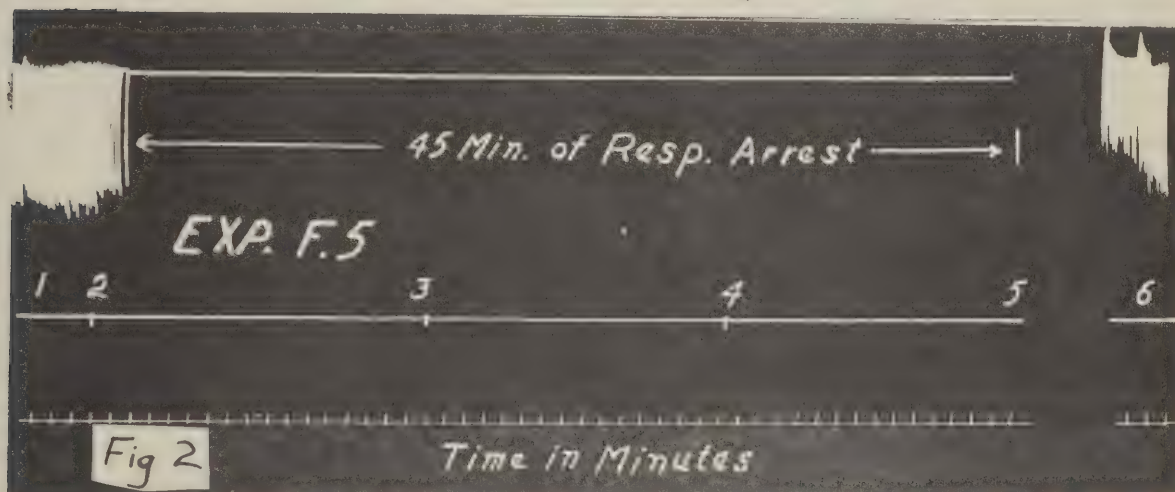
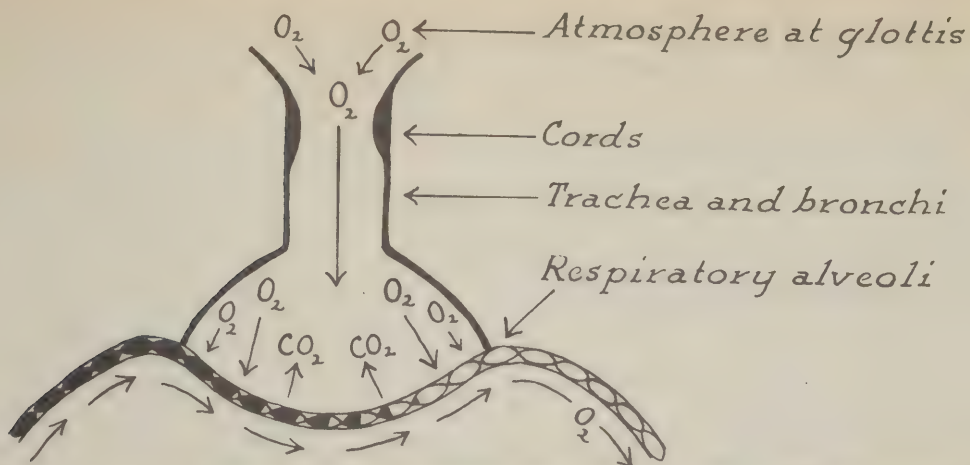


Fig. 2. Dog, male, weight 10.8 kg. Pneumographic record of respiration: 1. Control period: Respiration 13/min.; alveolar  $CO_2$  4.1%;  $O_2$  89.2%; venous pH 7.40. 2. Anesthesia deepened. 3. Fifteen minutes of diffusion respiration. 4. Thirty minutes of diffusion respiration: Venous pH 6.91. 5. Forty-five minutes of diffusion respiration: Alveolar  $CO_2$  49.1%;  $O_2$  34.1%; venous pH 6.78. 6. Return of spontaneous respiration; resuscitation required 5 minutes. Dog alive and normal in all respects two years later.



from the spirometer bell is almost unaffected by cessation of the respiration. This figure is of particular interest because it shows that the uptake of oxygen is reduced with the onset of circulatory failure and that with cardiac arrest the uptake of oxygen abruptly ceases. The ability of the "hemoglobin-oxygen pump" to exert a definite inward suction upon the atmosphere was also demonstrated in a second series of experiments in which the spirometer bell was seized during respiratory arrest and the resulting pressure changes within the bell recorded by means of a water manometer (fig. 4). As soon as the bell was prevented by seizure from falling during respiratory arrest, a negative pressure developed within the bell which, in one experiment, reached the level of 14.0 cm. of water before death. Post mortem examination of the dogs in this series showed in all cases a marked atelectasis of the lungs. These experiments demonstrate the danger of an obstructed airway during diffusion respiration.

It has been suggested by Comroe and Dripps that the small intermittent back-and-forth oscillations of the intrapulmonary gas generated by vigorous cardiac contractions is the motivating force in diffusion respiration and not the "hemoglobin-oxygen pump" postulated by us. It seems impossible that the steady progressive development of negative intrapulmonary pressure shown in this figure could have been produced in the manner they suggest.

In a third series of experiments the inability of the oxygen pump to maintain an adequate alveolar tension of oxygen when nitrogen is present in the atmosphere at the glottis was demonstrated in the following manner: A series of 10 dogs were placed within an oxygen chamber and subjected to forty-five minutes of diffusion respiration. The alveolar concentration of oxygen in 6 at the end of the arrest was 28.8%. The remaining 4 of these dogs were removed from the oxygen chamber at the conclusion of the experiment and placed in room air for 3 minutes. Very shortly after exposure to room air the tongue turned cyanotic and at the end of the three minutes all four dogs appeared near death. Samples of alveolar air taken at this time showed that the average concentration of alveolar oxygen had fallen to 9.9% and that the alveolar nitrogen had increased to a corresponding degree.

Behavior of Alveolar Gases during Diffusion Respiration. In this series and in subsequent experiments, the dog's head, only, was placed in an oxygen chamber. Six to 8 liters of oxygen per minute was run into the chamber. A free exit for excess gas, however, was provided by a low resistance flutter valve which kept the pressure within the chamber below 2 mm. of water. This slight positive pressure served the purpose of reducing the seepage of atmospheric nitrogen through the neck collar into the chamber. Although a slight positive pressure at the glottis favors the inflow of oxygen during respiratory arrest, it is not at all essential for successful diffusion respiration. We used a head chamber in this and subsequent experiments in order to allow free access to the body of the dog for the withdrawal of blood, etc. Respiration was recorded by a chest pneumograph (fig. 2).

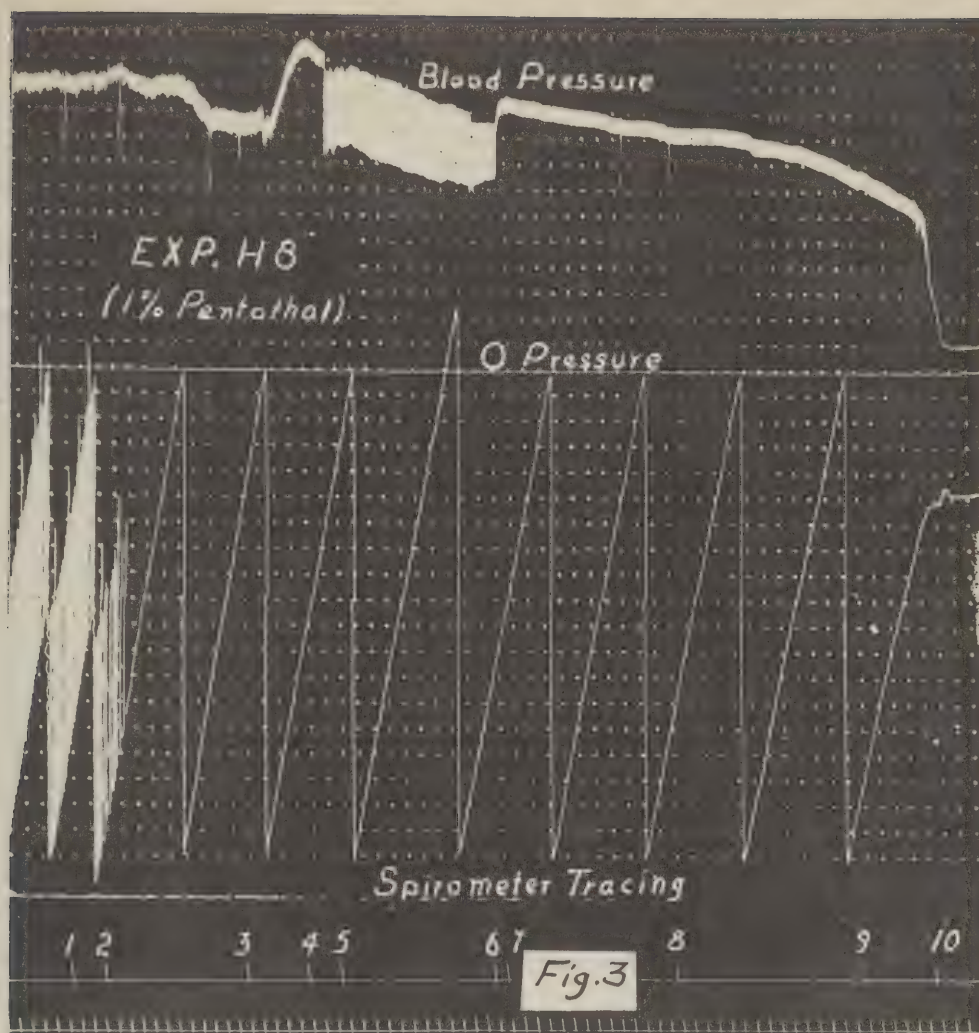


Fig. 3. Dog, male, weight 12.5 kg. Spirometer record of  $O_2$  uptake. Space between horizontal dotted lines = 35.1 cc. of  $O_2$ ; downstroke represents refilling of spirometer bell. 1. Control period:  $O_2$  uptake 8.2 cc./min./kg. 2. Anesthesia deepened. 4. Fifteen minutes of diffusion respiration:  $O_2$  uptake 7.9 cc./min./kg. 6. Thirty minutes of diffusion respiration:  $O_2$  uptake 7.2 cc./min./kg. 8. Forty-five minutes of diffusion respiration:  $O_2$  uptake 7.2 cc./min./kg. 10. Sixty-six minutes of diffusion respiration:  $O_2$  uptake 6.0 cc./min./kg. Note:  $O_2$  uptake decreases as the circulation fails and ceases abruptly with cardiac arrest.



The results obtained from 12 dogs show that the diffusion of oxygen inward during diffusion respiration is relatively adequate but that the diffusion outward of carbon dioxide is slow and that the concentration of this gas rises in the alveoli during respiratory arrest at the rate of approximately 1% per minute. The average alveolar concentration of oxygen at the end of 45 minutes of arrest in this series was 28.3% and that of carbon dioxide 54.7%.

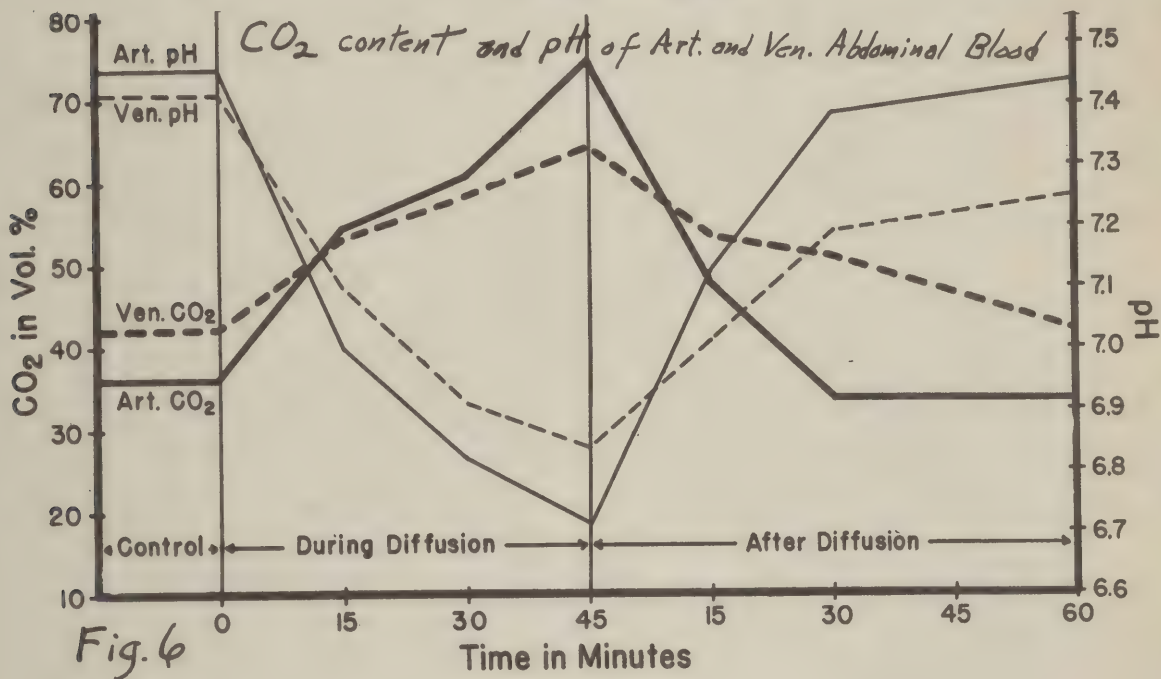
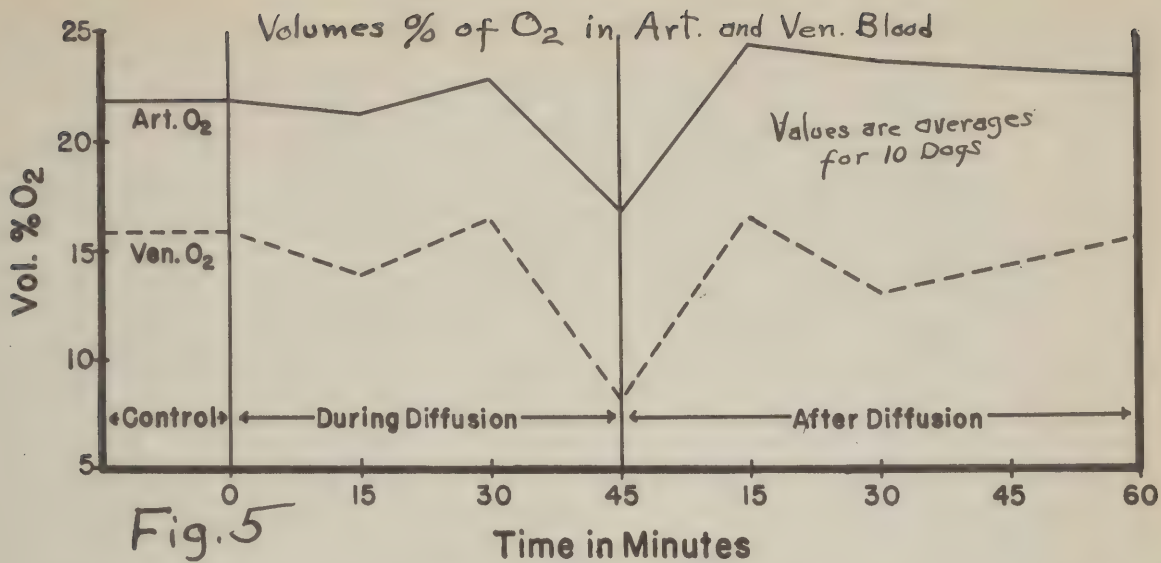
Survival from 45 Minutes of Diffusion Respiration. In one series of 12 dogs resuscitation was successful to the extent that spontaneous respiration reappeared in all 12 animals. One dog died 96 hours later from an internal hemorrhage. The remaining 11 made a complete and permanent recovery. No evidence of any disturbance in their behavior, appetite or appearance was detected. The average time required in this series for resuscitation to the resumption of spontaneous respiration was 4.8 minutes. In a subsequent series, following the adoption of an improved technique, the resuscitation time fell to an average of 47 seconds.

Oxygen and Carbon Dioxide Content and the pH of the Arterial and Venous Abdominal Blood during Diffusion Respiration. Abdominal arterial and venous blood was withdrawn at standardized intervals by means of intratracheal catheters passed up the femoral vessels to a distance of 15 cm. above the femoral (Scarpa's) triangle. Heparin was used as an anticoagulant. The oxygen and carbon dioxide content were determined by the micro method of Roughton and Scholander and pH by the Beckman Model G apparatus, using a closed glass electrode.

a. Oxygen Content of the Abdominal Blood. In 10 experiments the oxygen content, particularly of the arterial blood, was well maintained until the thirtieth minute of respiratory arrest. After this time the oxygen content of both arterial and venous blood progressively declined, due chiefly, perhaps, to the Bohr effect produced by low pH (fig. 5).

b. Carbon Dioxide Content and pH of Abdominal Arterial and Venous Blood. The pH progressively fell and the carbon dioxide content progressively rose as the arrest continued (fig. 6). Within the first 15 minutes of diffusion respiration a reversal occurred in the normal relationships between the carbon dioxide content and pH of venous abdominal blood and that of the arterial, i.e., the carbon dioxide content of the arterial blood became higher than that of the venous blood and concomitantly the pH of the arterial blood became lower than that of the venous. The average pH values at the end of the 45 minute of respiratory arrest were 6.72 for arterial and 6.84 for venous blood. Following resuscitation by intratracheal insufflation of oxygen the normal arterio-venous relationships between these values were restored. This paradoxical condition and its reversal occurred in each of 10 consecutive experiments.

Secretion of Urine under Diffusion Respiration. The urine during diffusion respiration was collected by means of a ureteral catheter passed up the ureter into the pelvis of the kidney. In 12 dogs a complete or nearly complete anuria occurred either simultaneously with the cessation of





respiration or within a few minutes later. The systemic blood pressure was adequate for glomerular filtration throughout all experiments. Urine secretion was resumed in an average of 1 minute 12 seconds after the resumption of spontaneous respiration. The intravenous injection of large quantities of 10% glucose in Ringers failed to break through the anuria.

The Circulation under Diffusion Respiration. It has been our custom to inject pentothal sodium rapidly during the first 15 minutes of respiratory arrest and to continue the injection progressively more slowly for the second 15 minutes. After 30 minutes of diffusion respiration no additional anesthetic was administered because from this time on the autogenous carbon dioxide can be relied upon to maintain respiratory arrest. The rapid injection of pentothal sodium during the early phase of diffusion respiration was in most instances accompanied by a considerable fall in blood pressure. From the 15th minute onwards the blood pressure commonly recovered and remained at approximately the control level for the remainder of the experiment. The early initial fall in blood pressure can be avoided by injecting the anesthetic at a slower rate.

A similar correspondence between the electrocardiogram and the rate of injection of pentothal sodium was observed. In 6 of 11 animals, ventricular extra systoles associated with a wide pulse pressure and a reduced diastolic pressure occurred at some time during the first 30 minutes of arrest. With the discontinuance of the injection of pentothal sodium and the coincident building up of autogenous carbon dioxide to a high level these extra systoles, in every instance, disappeared. It seems fair to conclude from this that the extra systoles were caused by high levels of pentothal sodium and not by the acidosis. The only other change in the electrocardiogram observed during diffusion respiration was a progressive increase in the amplitude of the T wave. An increase in the amplitude of the T wave has been previously reported by others as characteristics of acidosis. On the whole, the heart and circulation appear to withstand remarkably well a carbon dioxide acidosis of the degree of + or - 6.75.

Electrical Activity of the Brain in Dogs under Diffusion Respiration. A preliminary study of the electrical cortical activity of dogs subjected to diffusion respiration is underway. To date, 12 animals have been used. The control observations are made under light pentothal sodium anesthesia. Diffusion respiration is then maintained for 45 minutes in each animal. Electrode implantation is essentially that described by Hoagland (Science 1940). An Offner four-channel electroencephalograph is used. The animals are sacrificed 2 months after diffusion. Gross and microscopic examinations of the brain and other organs are being made.

The brain waves seen in the early minutes of diffusion respiration cannot be distinguished from those seen in deep pentothal anesthesia. However, the waves shortly become slower and of less amplitude. This condition is followed shortly by brief periods of absence of cortical activity. During the last 30 minutes of diffusion

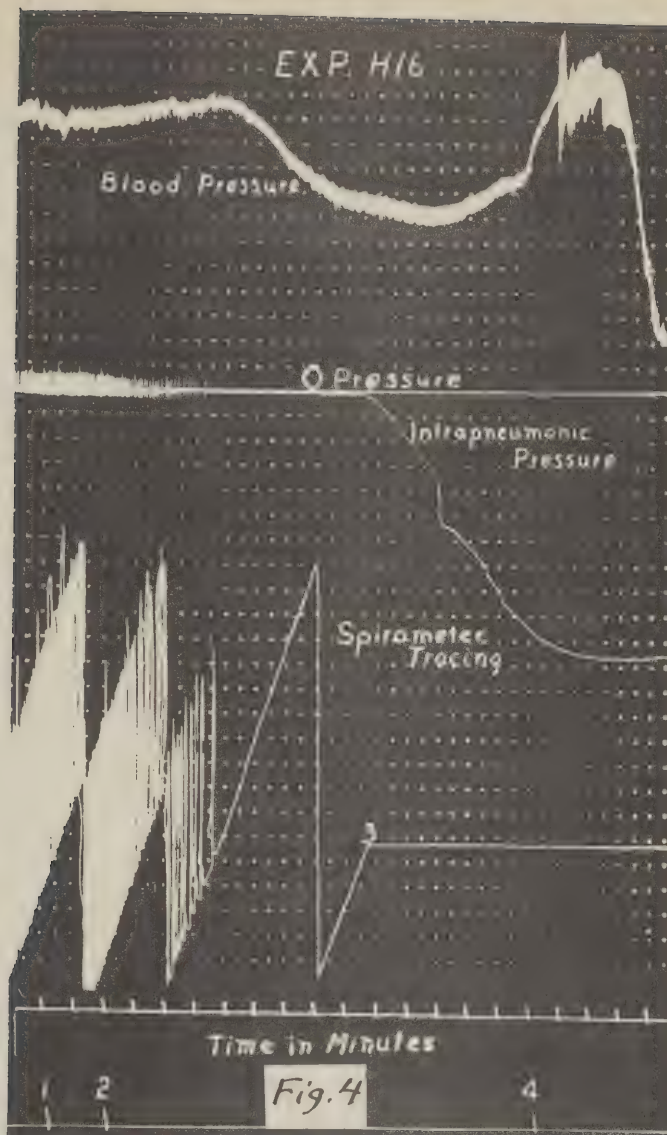


Fig. 4. Dog, male, weight 17.3 kg. Development of negative pressure within spirometer bell (intrapneumonic pressure) on seizure of bell. 1. Control period. 2. Anesthesia deepened. 3. Spirometer bell seized. Death 11 minutes after seizure of bell; maximum negative intrapneumonic pressure developed was 10.5 cm. of water.



respiration, in all animals studied so far, there is an almost complete absence of electrical activity of the cortex. Within a few moments after resumption of respiration, electrical cortical activity is again seen. Within a few days the electroencephalographic record appears indistinguishable from that obtained during the pre-diffusion period.

# USE OF THE LILLY NITROGEN METER IN THE NITROGEN ELIMINATION

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The best studies in recent years on gaseous nitrogen eliminated from the body tissues are those carried out by Behnke and his associates. Boothby, Lovelace and Benson in 1940 reported to the Air Surgeon some of the experiments carried out in this laboratory which were first published in "Physiology of Flight," Wright Field, 1940-1942. The data then presented were obtained by rebreathing (after washing out the pulmonary nitrogen with four deep breaths of oxygen) through soda lime into a series of bags containing oxygen; the final volume was carefully measured and analyzed for nitrogen content. The data were presented on semi-log paper; later when replotted on double log paper this data which was the average of 133 determinations from 17 runs on two subjects fall essentially on a straight line; the data of single runs fall even more closely to a straight line. Further analysis of the physiologic significance of nitrogen elimination was postponed till improved methods could be devised after the war.

Bateman, Boothby and Helmholtz again attacked the problem but the work was interrupted when Dr. Bateman accepted a research position with the Chemical Warfare Service. However, it was found that in normal subjects the pulmonary gaseous nitrogen was usually eliminated in  $2\frac{1}{2}$  to  $3\frac{1}{2}$  minutes with the subject breathing normally and that the rate of the accumulated preliminary nitrogen then made a sharp angle when plotted on double log paper with the accumulating body nitrogen.

It seemed then that this type of plot was well suited to differentiate the accumulated nitrogen that was essentially all from the lungs and air passages from that which was essentially all from the body tissues. Experiments were carried out by inhaling pure oxygen from a demand valve and expiring into a specially designed Krogh type of spirometer which accurately recorded volume changes. The dead and residual spaces of the apparatus were small and it was possible by means of a formula developed by Bateman to calculate the physiologic dead space and the functional residual air. Because of the limited capacity of the gasometer the open circuit type of experiment had to be limited to 8 to 12 minutes. This was a marked disadvantage for certain types of pathologic cases in which the pulmonary nitrogen elimination time was much prolonged (6 to 10 minutes).

Therefore, Boothby, Lundin and Helmholtz modified the recording respirometer by converting it to a closed circuit system with absorption of CO<sub>2</sub> by soda lime. At first the analyses for oxygen were made in the usual way after obtaining 12 samples in evacuated tubes. This however was time consuming and also not a sufficient number of air samples could be thus obtained to establish as accurately as desired the exact shape of the accumulated nitrogen curve.



Through the courtesy of the Navy we obtained a loan of a Lilly-Anderson nitrogen meter. Dr. Baldes suggested spreading the lower part of the scale so that readings could be obtained every 0.2 minutes if desired with as great an accuracy as obtainable by volumetric analysis by the Haldane method (below 10 per cent nitrogen with an accuracy to three significant figures ( $\pm 0.02$ )). The Lilly-Anderson nitrogen meter unfortunately cannot be calibrated in absolute terms. It is necessary to determine the zero setting on the "pure" oxygen in the spirometer saturated or nearly saturated with water vapor and circulating through the soda lime at a rate of 80 to 100 liters per minute; a sample collected over mercury analyzed by the Haldane method usually falls between 0.3 to 0.4 per cent inert gas of which about one-half is nitrogen and the other half argon. (Nearly "pure" argon causes hardly a perceptible change on the nitrogen meter). At the end of the experiment another sample is analyzed by the Haldane apparatus in duplicate for its nitrogen content. Calibration curves show that up to 20 per cent nitrogen (and probably considerably higher) the Lilly-Anderson nitrogen meter reads linearly with the nitrogen per cent as determined by a Haldane analyzer and to the same degree of accuracy when samples are obtained from a circulating closed system with temperature changes not exceeding  $1.5^{\circ}\text{C}$ .

The present system makes possible the determination of the rate of nitrogen elimination of normal subjects and patients with accurate determination of the lung washing out period. These data plus observations of vital capacity and its component parts allow estimations of functional residual volume, residual volume, rate of washing out of lung air and estimation of variations in rate during this period. These estimations help in assaying the efficiency of ventilation. In addition, the rates at which body nitrogen is eliminated is a resultant of factors of blood flow, capillary permeabilities, tissue content and so forth, and possible uses of these data are being investigated.

At the present time this method is proving useful in two respects. First, it is possible to obtain, without excessive effort, estimates of components of the total lung capacity which are of clinical value in such cases as gradually increasing compensatory emphysema, and in other cases where a decrease in vital capacity may signify decreasing total capacity, or increasing residual volume. Secondly, though as yet incompletely worked out, the characteristics and length of time of the lung washing out period, is proving of significance as an estimate of the efficiency of normal ventilation of the lungs at rest. It has also been possible to estimate the rate at which the venous blood is returning to the lungs from the tissues which of course is influenced to a variable and as yet undetermined amount by short circuits. These estimations are made at present in anticipation of the time when nitrogen content of mixed venous blood can be measured in conjunction with the test.

# OBSERVATIONS CONCERNING THE PATHOLOGICAL PHYSIOLOGY

## UNDERLYING EXERTIONAL DYSPNEA IN THE DISEASE

### "GRANULOMATOSIS OCCURRING IN BERYLLIUM WORKERS"<sup>1</sup>

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This recently discovered clinical entity (1) may at times be evidenced only by roentgenological changes in the lung (2). More often, however, symptoms are present and may vary in severity from slight to fatal. The typical symptoms of those in the active or "sick" phase of this disease are severe weight loss and weakness, severe and at times intractable non-productive cough, and severe crippling exertional dyspnea. The latter symptom is most striking and is the subject of the present study.

The data from several cases will be presented. From the comprehensive protocol of study the following measurements bear upon the pathological physiology of respiration: recumbent lung volume (3), lung ventilation efficiency (4), maximum breathing capacity (5), arterial blood gases and alveolar air gases at rest and during exercise. In addition minute ventilation and oxygen consumption were studied during exercise of increasing intensity on motor driven treadmill. Each walk was of six minutes duration, the expired air being collected during the fifth and sixth minute. The speed of the treadmill was chosen on the basis of each subject's anticipated capacity to exercise, and each man walked on progressive grades until the one at which he was unable to finish the six minute period was reached, the last grade being considered the peak of capacity of the individual to perform this type of work. Pulse and respiratory rate during exercise and recovery was observed. Oxygen debt studies at one grade were also made. Arterial blood samples were drawn during exercise on one of the high intensity grades by means of an indwelling needle.

Figure 1 shows the relation of minute ventilation to oxygen consumption at varying grades of exercise. The response of a normal male (age 39) walking at stints from a level grade to a 16% grade, progressing in 2% increments, is indicated by #9. It shows the normal linear

<sup>1</sup>This study is supported by research grants from: a, U. S. Navy, Office of Naval Research, b, U. S. Public Health Service



relationship between ventilation and oxygen consumption until the higher grades are reached, at which time minute ventilation continues to progress by normal increments, whereas oxygen consumption does not keep pace. The peak of oxygen consumption is reached at 310 cc oxygen/minute. (It is generally accepted that the maximum of ability to supply oxygen to the body economy measures the peak of capacity for physical energy output.) Numbers 1, 2, 3, 4, 5, 6, 7, and 8 are bona fide cases of granuloma of the lung occurring in beryllium workers, no. 1 being the same individual as no. 8 but studied after an interval of 20 months, during which time a severe deterioration in his physical condition had developed. Their inability to attain a high level of oxygen utilization is strikingly demonstrated. Moreover, the degree of clinical disability is perfectly in accord with their determined physiological limitation for oxygen utilization, Numbers 1, 2, 3, 4, and 5 being clinically more disabled than 6, 7, and 8. No. 10 is a granuloma suspect and no. 11 is a bona fide case of Boeck's Sarcoid in a slightly active phase with extensive abnormal parenchymal shadows in the lung roentgenogram. The mechanism underlying the inferior capacity of this group (nos. 1 through 8) to utilize oxygen can be sought in one or a combination of three factors. The tissues may be intrinsically unable to utilize oxygen - a metabolic failure. Inadequate blood flow, abnormality of hemoglobin or low arterial  $pO_2$  may cause a failure in delivery of sufficient quantities of oxygen to the tissues - a transport failure. Some impediment other than failure of oxygen supply or utilization, as for example, muscle weakness or exertional dyspnea, may prevent the individual from reaching intensities of work high enough to utilize the maximum supply and metabolic capacities of the body.

Data not published in this report, showing that oxygen utilization per kilogram meter of work done is within the realm of normal, and also that oxygen debt studies are normal, strongly indicates that the muscles are entirely capable of metabolizing oxygen.

The following observations are pertinent to adequacy of oxygen supply. Anemia is not a factor, in fact a polycythemia is quite common and characteristic of this disease. There have been no observations concerning cardiac output or blood flow in the tissues and, therefore, it is impossible to make any comment concerning a failure of adequate oxygen supply on this basis. Tables 3 and 4 reveal that there has been, in most of these cases, a definite unsaturation of arterial hemoglobin at rest and during exercise. In some, this is a very prominent feature. In one typical case, however, (who has been restudied) there is no polycythemia and no arterial unsaturation. This is no. 7 on figure 1. Whereas hemoglobin saturation may be pathologically low, nevertheless because of the polycythemia, the oxygen content of the arterial blood is usually within normal range. It is true, however, that the oxygen tension ( $pO_2$ ) is lower than normal in all and very low in some. It is possible, therefore, that the transfer of oxygen from the blood to tissues may be somewhat impaired and, at high rates of demand, a failure of supply may thus occur. The abnormality of  $O_2$  tension may be reflected more directly and to a greater degree in the mechanism underlying exertional dyspnea.

Further search for an explanation of the limitation of this group's ability to attain a high oxygen utilization leads to the complaint of muscle weakness and exertional dyspnea. Either one of these symptoms might develop at such low levels of physical exertion that the individual would find it impossible to force himself to high oxygen utilization. A pertinent observation should be made about the limiting feature of exercise on the treadmill in these cases in contrast to that in the normal individuals we have studied. Generally, and almost without exception, the normal man finds that muscular weakness or an inability to make his legs continue to work determines his upper limit of physical energy output. In other words, the limiting factor is muscle failure. Although at this point, breathing may be labored, the individual does not "give up" on the treadmill because of extreme shortness of breath. In contrast to the fact that muscle weakness limits the normal man in going to a maximum effort, these pathological subjects note that extreme shortness of breath is the factor that makes them "give up" on the treadmill. In some there has also been a feature of leg weakness, but by far the predominant and most distressing feature limiting their ability to exercise is the development of shortness of breath. We define exertional dyspnea as "the awareness of respiratory distress". One can conceive of the respiratory apparatus as being a pump which moves air at a variable rate depending upon the circumstances. As the load placed upon this pump utilizes increasing percentages of its capacity, the sensations set up by the action of the pump become stronger. Although these sensations at rest and during moderate grades of exercise are ignored or do not reach consciousness, at higher intensities they become strong enough to force the individual to be aware of them. This is the factor of awareness of respiration. If the circumstances under which the "awareness of breathing" sensation occurs are normal, one does not become distressed. It is only when the awareness of these sensations occurs under conditions that should not produce them, that one becomes distressed. In other words, shortness of breath is a perfectly normal phenomenon when the strain is such that it should occur. When, however, shortness of breath occurs at levels of strain that should not produce shortness of breath, then the sensation becomes distressing. In these pathological subjects, sensations of breathing appear at levels of exertion that did not previously bring them forth and would not bring them forth in the normal man. Therefore, these men can correctly be considered as suffering from exertional dyspnea. This conception of the problem of dyspnea can be explored mathematically if one has a way of measuring load and capacity. Previous investigators, including this laboratory, have chosen to measure load in terms of minute ventilation at 37° C. ambient pressure. Capacity is measured in terms of maximum breathing capacity per minute as originally defined by Hermannsen. The ratio is expressed:

$$\frac{\text{Minute Ventilation}}{\text{Maximum Breathing Capacity}}$$

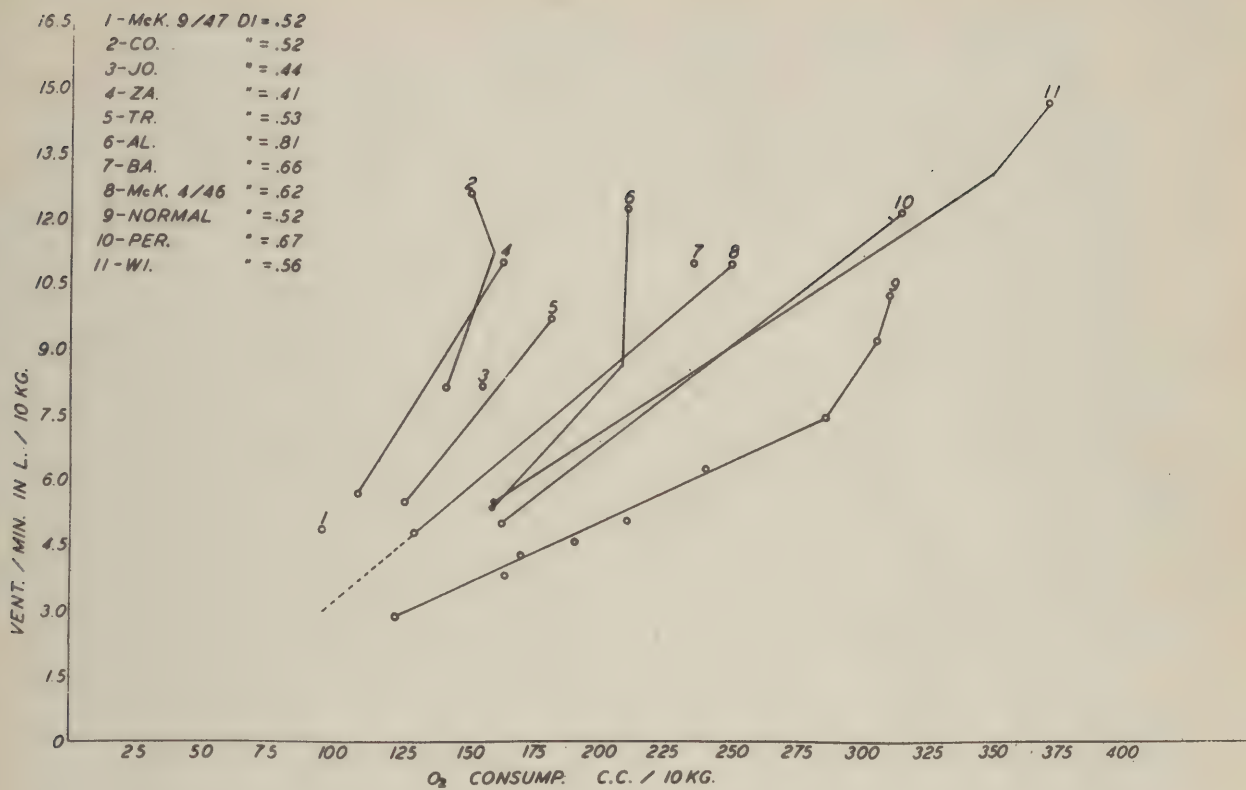
In general, in normal individuals, one begins to be aware of breathing when the minute ventilation constitutes 30% to 35% or more of the breathing reserves (maximum breathing capacity). It is obvious that this percent of utilization of breathing reserves could be reached at lower than normal levels of exertion in one or a combination of two ways. The load could be high in proportion to the energy expenditure; in other words, minute ventilation could be high for the level of work



being done, thus bringing about a utilization of a larger than normal percent of breathing reserves - a disproportionate increase in the numerator. A subnormal maximum breathing capacity will likewise affect the ratio in the same sense, by reducing the value of the denominator. Either of these two changes could alone or in combination lead to the utilization of a larger than normal percent of breathing reserves at a given level of exercise. A study of these factors has been made. A third factor that must be considered is the sensitivity of the cortex, or the ease with which breathing sensations are perceived. If the sensitivity of the cortex is high, one may perceive breathing sensations more acutely and severely than when the breathing sensitivity is normal or depressed. This may in some produce exertional dyspnea or distressing breathing at lower than normal levels of exertion, even though "load" and "capacity" are normal. This has not been the case, to a notable extent at least, in any of the subjects that we are now discussing. It is worthwhile to note that in table 1, the dyspnea index (Minute Ventilation/Maximum Breathing Capacity) is within a normal range at the height of capacity for physical energy output. In other words, all of these individuals were able to force themselves to use a high percentage of their breathing reserve; in fact, some were able to force themselves to use a higher percent than the normal individual could. In table 1, it is apparent that the determined maximum breathing capacity is higher, equal to or only slightly lower than the predicted maximum breathing capacity, in all except two, McK and Ma. It is important to note that McK showed a marked drop in determined M.B.C. over a twenty-one month period of physical deterioration. That the loss of M.B.C. is associated with a loss of lung volume or the development of emphysema is apparent from the data in table 1, which reveals that with the exception of Ba, those having a low M.B.C. also had either a low total volume or a high percent of residual air - the latter suggesting the presence of extensive pulmonary emphysema. Two of the cases, Co and Za, experienced extreme exertional dyspnea in spite of having very high breathing reserves. It is apparent, therefore, that although in some a loss of maximum breathing capacity contributed to exertional dyspnea (in two, McK and Ma, this was a very important factor), in the majority of the cases it would not serve in any way as an adequate explanation of this severe symptom.

In contrast, an examination of the response to exercise in terms of minute ventilation shows a strikingly different picture. Table 2 gives data concerning the response to exercise at the peak of effort. It is apparent by inspection that the minute ventilation is extremely high for the intensity of exercise in the pathological subjects as compared to the normal individual. Careful attention must be paid to the level and speed of treadmill in making this comparison. A better way to study this factor is by means of the ventilation equivalent,  $O_{2V}$ . If one assumes that energy expenditure is directly related to oxygen consumption, it is permissible to express minute ventilation in terms of liters of air breathed per liter of oxygen utilized. As noted in table 2, it is clear that the pathological subjects breathe two to three times as much air for their energy expenditure as does the normal man. This factor contributes very largely to the abnormally high dyspnea index at fairly low levels of energy expenditure

FIGURE I





in these subjects. That it is related in some way to oxygen is apparent (table 2) from the fact that when respiring pure oxygen during exercise of the same intensity on the treadmill, the minute ventilation was markedly reduced in all of these pathological subjects as compared to the reduction in the normal man.

It is beyond question, therefore, that in these cases, overbreathing or an increase in the numerator is a primary factor in the development of exertional dyspnea and in two of the cases, it is the sole factor. A loss of breathing reserve is also a very important added factor in two cases.

The mechanism whereby overbreathing occurs has not been completely explored. That it has something to do with oxygen is apparent from the data in table 2. Examination of arterial blood obtained at rest and during exercise (table 3) reveals that unsaturation of the hemoglobin is a consistent finding, with the exception of one case (Ba). This suggests that the oxygen tension in the arterial blood is lower than normal. Complete studies of arterial oxygen and CO<sub>2</sub> tensions and oxygen gradient (6) of two cases are shown in table 4. The extremely low blood pO<sub>2</sub> at rest and during exercise in both of these cases is striking and almost certainly contributes to the phenomenon of overbreathing exhibited by these two individuals. It is apparent that in one, Co, there is no retention of CO<sub>2</sub> because the blood pCO<sub>2</sub> is low both at rest and during exercise. In the other, however, there is a retention of CO<sub>2</sub>. It is of interest that the first case has no concomitant pulmonary emphysema, whereas the second has a very severe grade of pulmonary emphysema. The arterial gradient, both as calculated by the method of Riley and Lilienthal and as actually measured by the Haldane method, is very high in both cases.

### Discussion

It would appear that the inability of these pathological subjects to push their oxygen consumption to normal high levels is due, primarily, to the fact that exertional dyspnea develops at such low levels that each individual is unable to exercise his muscles sufficiently to reach high levels of oxygen consumption. The exertional dyspnea seems to be due to two of the three factors discussed, in view of the fact that overbreathing in response to exercise (an increase in the numerator) and a loss of breathing reserves (a decrease in the denominator) are found to occur. The phenomenon of overbreathing is associated at least in part with a low arterial oxygen tension. Without exception, in the cases exhibiting exertional dyspnea, a low arterial oxygen tension has been found, both at rest and (particularly) during exercise. In some, this has been more marked than in others. It is postulated that the low oxygen tension acting by way of the chemo receptors, stimulates the respiratory center to abnormally great activity. It is also possible that low oxygen tension may act locally in the exercising muscles to bring about the same phenomenon in a reflex manner. Loss of breathing reserve (M.B.C.) is due in part to loss of lung substance in certain of the individuals, and is also due to the

development of pulmonary emphysema in others. The autopsy material thus far shows a very high grade of pulmonary emphysema and also marked loss of lung substance.

From our data, it would appear that chronologically, the earliest pulmonary lesion in some way changes the gradient for the passage of oxygen across the alveolar membrane with a resultant abnormally high gradient and low  $pO_2$  in the arterial blood, which in turn leads to overbreathing, and the development of exertional dyspnea. As the disease progresses, lung tissue is destroyed, and pulmonary emphysema develops, with a consequent loss of breathing reserves further compounding the abnormality of breathing and accentuating the severity of exertional dyspnea.

It should be noted that in our experience, these cases are very exceptional in the high degree to which interference with passage of oxygen from alveolar air to arterial blood occurs. The large gradient between alveolar air and arterial blood  $pO_2$  is almost certainly due to an alveolar membrane abnormality. Histological examination of the tissues of the lung from two cases studied during life in this laboratory shows extensive thickening of the alveolar walls with adjacent capillaries that are still patent. Thickening of the alveolar walls occurs as part of the generalized fibrosis of the lung in other diseases but we have never observed a similar high gradient in such diseases, as for example silicosis, disseminated tuberculosis or disseminated pneumonitis. In these latter diseases, the vascular bed is for the most part destroyed in those areas that are the site of the alveolar wall thickening and hence there is no opportunity for a high gradient to develop. Although poor ventilation of lung segments - as in emphysema or bronchial obstruction - can produce a low arterial  $pO_2$ , such a mechanism does not play the dominant part in the cases under discussion. Two of the most striking examples of low arterial  $pO_2$  in the series (Co and Za) had no evidence of emphysema or poor ventilation of the lung.

### Summary

1. Overbreathing, especially during exercise, and loss of breathing reserve are the primary causes of exertional dyspnea in "Granulomatosis occurring in Beryllium workers". Overbreathing is clearly the earliest, predominant, and sometimes the sole cause of the dyspnea. Loss of breathing reserve is a late development and severely augments the dyspnea.

2. A low arterial blood  $pO_2$  and higher than normal gradient for oxygen across the alveolar membrane was observed in each case of granulomatosis. The possible role of this factor as a cause of overbreathing is discussed.

3. Loss of total lung volume and the development of emphysema was observed in several cases, and the role of these factors as a cause of loss of breathing reserve is discussed.



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Table 1.

Case	M. B. C. Determined	M. B. C. Predicted	% Difference	Total Lung Volume Determined	Total Lung Volume Predicted	% Difference	Residual Air as % of Total Volume
McK	132	150	-12	4.77	4.92	-3	38
" 1	66	150	-56	4.94	4.92	0	60
Co	198	140	+41	3.63	4.87	-25	30
" 2	162	140	+16	2.98	4.87	-39	25
Jo	103	128	-20	3.26	4.45	-27	25
Za	178	157	+20	4.71	5.40	-13	29
Al	102	138	-26	5.47	4.77	+15	50
Ba	124	178	-30	6.08	5.99	+2	37
Ma	54	114	-53	2.79	4.95	-44	39

<sup>1</sup>21 month interval<sup>2</sup>15 month interval



Table 2.

Case	Miles per hour	Treadmill Grade %	Ventilation Liters/minute	Oxygen Consumption Liters/minute	Ventilation Equivalent O <sub>2</sub> V	Minute Ventilation/M.B.C.	Dyspnea	Ventilation Breathing Air	Ventilation Breathing Oxygen	% Drop in Ventilation
Norm.	3.5	8	51	1.9	27	.27	0	51	45	-12
McK	3.5	8.6	81	1.87	44	.62	4+	81	63	-29
" <sup>1</sup>	1.5	0.0	31	0.66	47	.47	3+	33	21	-24
Co	3.0	6.0	84	0.97	86	.53	4+	56	35	-34
Jo	3.0	0.0	44	0.86	52	.43	3+	-	-	-
Za	2.0	8.0	76	1.14	66	.42	3+	75	50	-33
Al	3.5	8.6	83	1.40	59	.81	4+	59	39	-34
Ba	2.5	8.6	82	1.76	47	.66	4+	82	51	-38

<sup>1</sup>21 month interval

Table 3. Arterial Blood Oxygen in Volumes %.

Case	During Rest			During Exercise		
	Oxygen Content	Oxygen Capacity	% Hemoglobin Saturation	Oxygen Content	Oxygen Capacity	% Hemoglobin Saturation
McK	19.9	23.6	84	17.1	24.6	70
Co	20.3	27.7	73	19.3	29.3	66
Jo	13.9	16.3	86	13.3	16.5	80
Za	19.0	23.4	81	-	-	-
Al	19.3	23.6	82	20.4	24.0	85
Ba	16.7	18.2	92	18.5	19.9	92
Ma	16.6	19.5	85	-	-	-



Table 4. "Gas Pressures Across the Alveolar Membrane"

	At Rest		During Exercise	
	Co	McK	Co	McK
Arterial Blood pCO <sub>2</sub> <sup>1</sup>	28	50	33	51
Arterial Blood pO <sub>2</sub>	45	60	43	43
Alveolar Air pO <sub>2</sub> (R-L) <sup>3</sup>	108	83	103	80
pO <sub>2</sub> Gradient	63	23	60	37
Alveolar Air pO <sub>2</sub> (Haldane)	108	76	-	105 <sup>2</sup>
Alveolar Air pCO <sub>2</sub> (Haldane)	28	49	-	31 <sup>2</sup>
O <sub>2</sub> Consumption Liters/min.	0.29	0.28	0.94	0.66
Hemoglobin Saturation	73%	84%	66%	70%

<sup>1</sup>Tensions are expressed in millimeters of mercury

<sup>2</sup>From terminal expired air by Henderson method

<sup>3</sup>Formula of Riley and Lilienthal

$$\text{Calc. alv. pO}_2 = \text{Trach. pO}_2 \frac{(\text{N}_2\% \text{ exp.})}{(\text{N}_2\% \text{ insp.})} = \frac{\text{pCO}_2 \text{ art.}}{\text{Exp. air R.Q.}}$$

# OBSERVATIONS REGARDING THE INTERPRETATION OF THE RESTING CALCULATED ALVEOLAR-ARTERIAL GRADIENT

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Fifty determinations of the resting alveolar-arterial gradient for oxygen have been carried out by the method of Riley et al.<sup>(1)</sup> on some 40 subjects. These included 8 beryllium workers, 4 silicotics, 3 cases of bronchial stenosis, 10 fibrotic tuberculous cases, and 7 normals. Striking results were seen only in the beryllium cases where the gradient was consistently very high; in the remaining diseased cases no consistent change was noted. In normal subjects, values varying from + 19 mm. Hg to -2 mm. Hg were observed and an attempt is being made to determine the cause of these rather marked variations.

Our subjects were not in a strictly basal state, nor was an indwelling needle used in most instances. The skin and peri-arterial tissues were well infiltrated with novocain before the puncture was performed and the procedure was alleged to be painless by the subjects. Despite this and the fact that the patients were put at ease, there was always the possibility that the slight sensations experienced may have changed the breathing pattern during the collection of expired air, thus bring about an "unsteady state" of respiration.

A correlation of the data from 40 cases has been made in some detail. Table 1, in which the entire group is divided into those above and below a gradient of 14 mm., shows the significant data relating to the calculated gradient.

Table 1.

	Low Gradients (18 cases)	High Gradients (22 cases)
Mean Arterial pCO <sub>2</sub>	42 mm.	37 mm.
Mean Expired RQ	0.73	0.80
Mean calc. Alveolar pO <sub>2</sub>	86 mm.	97 mm.
Mean Arterial pO <sub>2</sub>	79 mm.	76 mm.
Gradient Range	-2 to +14 mm.	+15 to +63 mm.
Ventilation equiv. (mean)	24 L.	31 L.



Several striking differences are to be noted between the two groups. The cases showing high gradients have, on the average, a considerably lower arterial  $p\text{CO}_2$  and a higher RQ. The nature of the equation (1) for determining effective calculated alveolar  $p\text{O}_2$  explains why this latter value averages 97 mm. for the high gradient cases and only 86 mm. in the cases with low gradients. It is of interest to note that a high ventilation equivalent is associated with a high gradient. By contrast, the average arterial  $p\text{O}_2$  is not strikingly different in the two groups.

The possible causes for these variations seemed important to investigate because in the same subject on different days and even within a period of ten minutes on the same day, similar variations were seen as indicated in Table 2.

Table 2. Normal Resting Subject.

Arterial $p\text{CO}_2$	RQ	Calculated al- veolar $p\text{O}_2$	Arter- ial $p\text{O}_2$	Gradient	Minute Volume S.T.P.D.	$\text{O}_2\text{v}$
37	.71	93	83	10	3.95	20
45	.77	85	78	7	6.30	24
40	.64	80	74	6	4.70	21
42	.75	86	83	3	4.15	20
47	.71	77	79	-2	5.45	22
<u>During hyperventilation</u>						
32	1.43	117	98	19	24.20	55
<u>47 seconds after end of hyperventilation</u>						
30	.48	82	82	0	1.17	3

It was further found that the expired air RQ of the individual subject was a very variable datum, since values such as those seen in the 4 examples of table 3 were often encountered when expired air was collected in carefully rinsed Douglas Bags for a period of two successive minutes before the artery was entered, during the time of blood collection, and for the minute after the needle was withdrawn. No consistent pattern of RQ rise and fall was found to occur in the individual or in the groups.

Table 3. Characteristic Resting RQ Changes  
from Minute to Minute

Sub- ject	2 min. before blood collec.	1 min. before blood collec.	During blood collection	During minute after blood collection
1	.69	.73	.77	.82
2	.79	.82	.72	.73
3	.82	.80	.84	.82
4	.86	.83	.88	.84

Correlation graphs were made between all the data that entered into the gradient equation with the addition of the ventilation equivalent ( $\frac{\text{Minute ventilation in liters}}{\text{O}_2\text{v} - \text{O}_2 \text{ consumption per minute in liters}}$ ) which is a rough measure of over- or under-ventilation.

a. In general RQ was inversely proportional to  $\text{pCO}_2$  but not in all cases.

b. RQ was found to be directly proportional to  $\text{O}_2\text{v}$  in most cases.

c. The calculated alveolar  $\text{pO}_2$  was fairly well correlated with  $\text{O}_2\text{v}$ , being directly proportional as a rule.

d. The arterial  $\text{pO}_2$  was found not to correlate with any of the data examined.

e. The most striking correlation was found between arterial  $\text{pCO}_2$  and the calculated alveolar  $\text{pO}_2$ , which were inversely proportional to each other. This is shown in figure 1. It is of interest to note that both normals and beryllium cases fall on the general slope described by the individual points. It can also be seen that the high gradients accompany a rise in calculated alveolar  $\text{pO}_2$ .

Discussion. These variations are evidence for the conception, previously discussed by Riley and Lilienthal<sup>(2)</sup>, that the Alveolar-Arterial gradient is probably made up of several components. The following components are tentatively considered to determine the total gradient.

a. Tissue-fluid barrier: presumably fairly constant in the same subject at rest.

b. Influence of venous admixture; the contamination of left ventricle blood by venous blood via the bronchial veins, the Thebesian system, pulmonary artery-vein shunts etc. would obviously lower the arterial  $\text{pO}_2$  and thereby increase the gradient. Failure



to reach a dynamic equilibrium between arterial and alveolar  $pO_2$  because of a high rate of capillary blood flow would also increase the gradient.

c. Gas barrier: this would include various respiratory changes affecting (differently?) the rate of flow of  $O_2$  and  $CO_2$  in the lung. In addition, if the expired air  $RQ$  is not identical (either actually or displaced in the "time phase" of a steady state) with the  $RQ$  of the gas to which the blood is exposed, a spurious barrier may be thought to be present. These changes might constitute an important component when  $RQ$  is changing from minute to minute, especially in cases of bronchial stenosis and emphysema, where there is unequal ventilation in various parts of the lung.

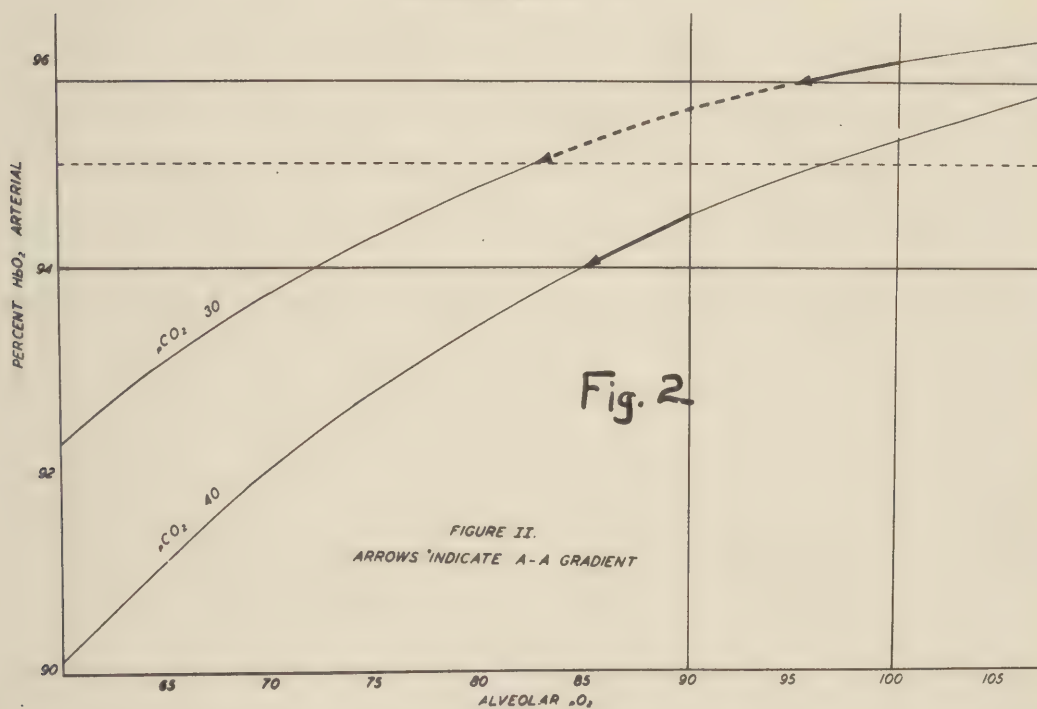
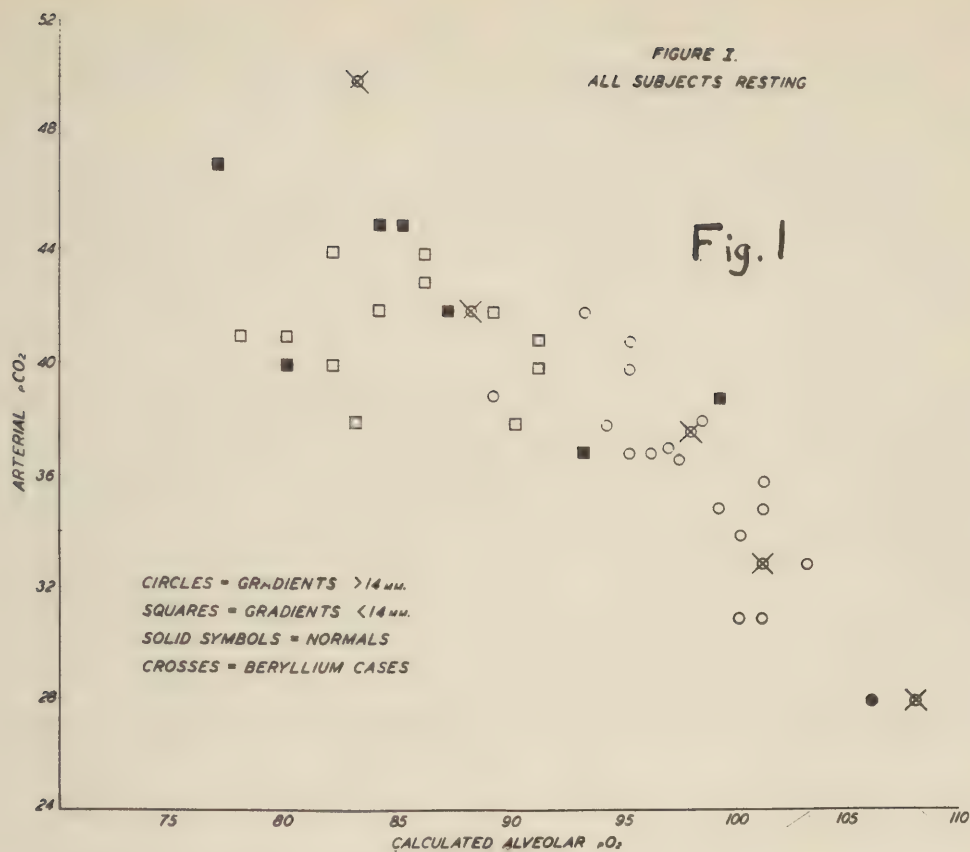
d. Factors involving the hemoglobin dissociation curves for  $O_2$  and  $CO_2$ : in figure 2, a hypothetical situation is illustrated in which hyperventilation raises the alveolar  $pO_2$  from 90 to 100 mm. and produces a fall in  $pCO_2$  from 40 to 30 mm. Under these circumstances, if the hemoglobin saturation is less than 99% and if there is to be no reduction in the A-A oxygen gradient of 5 mm., the percent saturation must rise 1.8% on the basis of the approximate hemoglobin dissociation curves illustrated. If, for any reason, the saturation rises but 1% (dotted horizontal line), the arterial  $pO_2$  might actually fall during hyperventilation (dotted arrow). This would increase the gradient from 5 to 17 mm., a variation of the order we have seen in the same normal "resting" subject at different times.

e. Red cell-plasma equilibrium: it is conceivable that further adjustment of the equilibrium between hemoglobin and physically dissolved plasma  $O_2$  may take place as the blood moves from the alveolar capillaries to the point of arterial sampling. This would be particularly liable to occur if the arterial  $pCO_2$  were low or changing. If such changes do occur while the blood is en route, the arterial  $pO_2$  as determined would be actually lower than it was in the alveolar capillaries, thus increasing the gradient.

Summary. In our hands the A-A gradient shows great variations which are not easily correlated with diseased states. From correlation studies it appears that the calculated alveolar  $pO_2$  is a function of the arterial  $pCO_2$ , being inversely proportional as a rule. It is believed that a need exists for some means of further differentiation between the various components which apparently combine in any given case to produce the total gradient.

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# CONTINUOUS ANALYSIS OF ALVEOLAR GAS COMPOSITION

## DURING WORK, HYPERPNEA, HYPERCAPNIA AND HYPOXIA\*

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One of our objectives during the last few years has been to describe the dynamics of the alveolar gas exchange as it is altered by hyperpnea, exercise, and changes in the oxygen and carbon dioxide concentrations of the environment. Two factors have greatly contributed to this reanalysis of classical respiratory physiology: a, a method for the continuous analyses of alveolar air (1); and, b, the development of an alveolar air and alveolar ventilation equation (2). The presentation of this respiratory data is greatly facilitated by the use of the O<sub>2</sub>-CO<sub>2</sub> diagram (2) which allows the simultaneous visualization of oxygen, carbon dioxide concentration in the lung, the respiratory quotient, the alveolar ventilation and hemoglobin saturation.

### Method

Since our first description of the continuous sampling of alveolar air we have been able to simplify our method so that this device can be easily constructed and installed into any breathing system (figure 1). A pump delivers continuously 150 ml/min. of alveolar air (the last 10 to 15 cc. of each tidal volume) to the automatic gas analyzers. Alveolar air is always present in the upper end of the expiratory tube except during the first phase of expiration. To prevent the dead space air from being drawn into the analyzer the alveolar air content of the balloon is pushed back into the main expiratory tube by the positive mask pressure of expiration. This volume far exceeds the pump capacity during this phase. Upon inspiration the balloon is again filled with alveolar air by the negative mask pressure.

The O<sub>2</sub>-CO<sub>2</sub> Diagram. Any alveolar value can be represented by a point when the O<sub>2</sub> is plotted against the simultaneous CO<sub>2</sub> tension. The computed respiratory quotients form straight lines originating at the inspired oxygen tension, while the lines of equal alveolar ventilation lie parallel to each other with a slope of  $-.209$  if air is

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\*Work done under contract with Air Materiel Command, Wright Field.



breathed. Thus the circle in figure 2 represents an alveolar air concentration at sea level. This automatically fixes the R.Q. as 0.85 and the alveolar ventilation (if the oxygen consumption is 250 ml) as 4.5 l/min. Any deviation of this point is brought on by alteration in one or more of these factors.

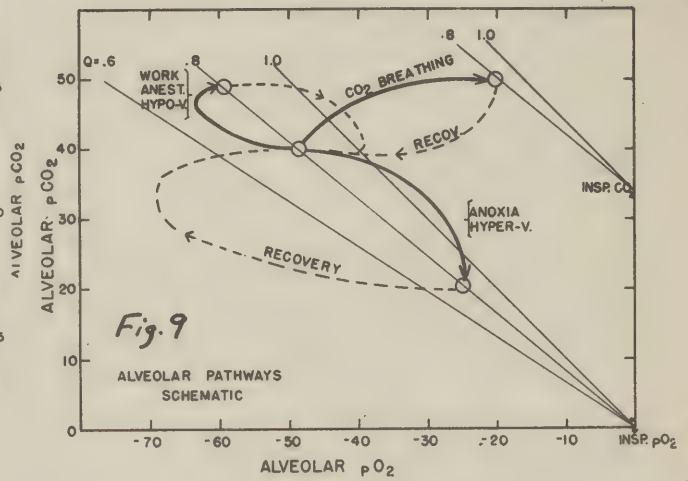
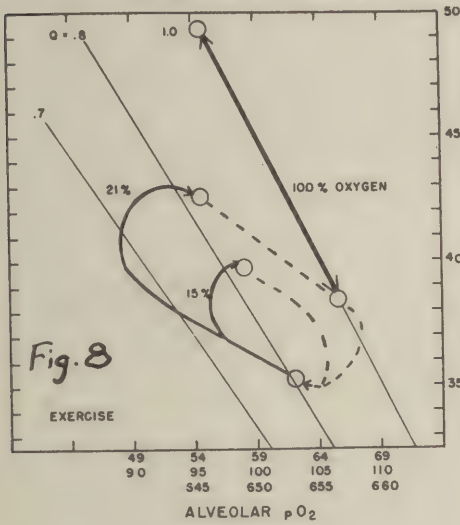
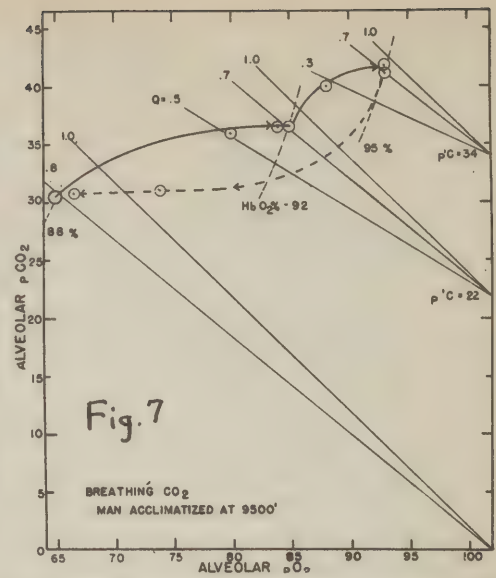
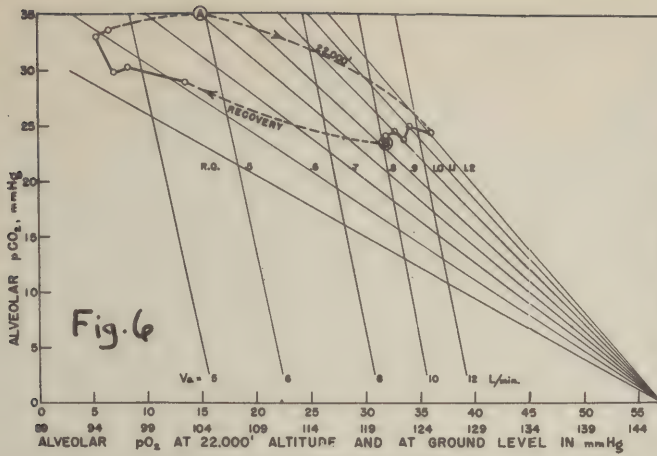
If one goes to high altitude one simply slides the R. Q. and ventilation lines along the abscissa until the origin of the R. Q. lines coincides with the new inspired oxygen tension. Thus in addition the simultaneous HbO<sub>2</sub>% saturation can be read off, if we assume that there is no appreciable gradient between the alveolar air and arterial blood. These iso-saturation lines are determined from the monogram of Henderson and Dill. The slope represents the Bohr effect.

1. The primary respiratory acidosis and alkalosis pathway. The circle in figure 3 represents an average alveolar air concentration. However, repeated determinations show that this point will vary from time to time as indicated by the normal range. This holds true not only in the resting state but also under strict basal conditions in trained subjects as Carpenter and Lee found many years ago (3). If the breath is held for increasing periods this curve can be extended to the left or after a few breaths of hyperventilation to the right as indicated. Thus acute changes in ventilation affect the gas exchange in a very precise manner largely by alterations in CO<sub>2</sub> output, the oxygen consumption tending to remain constant.

Thus far the initial shift of the normal alveolar air under various conditions (exercise, anoxia, hyperventilation, anesthesia, deadspace increase) has always been observed to follow one of these precise pathways. We might thus refer to these pathways as primary respiratory acidosis and alkalosis pathways which precede the secondary accommodations which will be described below.

It is of interest to speculate here about the so-called unequal gas exchange in the lung. This may be brought about by unequal blood flow or unequal ventilation of various parts of the lung. It is tempting to consider that the various alveolar gas concentrations that must exist, are represented by points lying along this pathway and that when combined give us so-called "normal" alveolar air.

2. Hyperventilation. If the alveolar ventilation is suddenly increased and maintained at 28 l/min. the alveolar air composition according to the ventilation equation must at any time lie somewhere along this iso-ventilation line. Figure 4 shows the minute by minute readings of four subjects. Prior to hyperventilation their alveolar pCO<sub>2</sub> was 37.5 mm Hg with an R. Q. slightly above 0.8 and an alveolar ventilation of 5.8 liters. With maintained hyperventilation for ten minutes the pCO<sub>2</sub> fell off along the iso-ventilation line, rapidly at first and cutting across the various R. Q. lines. After 10 minutes a pCO<sub>2</sub> of 16 mm. was reached with an R. Q. of about 1.3. If ventilation had been maintained beyond this time the steady state would have been reached at a pCO<sub>2</sub> of 9 mm Hg where this particular iso-ventilation line intersects the original R. Q. line of 0.8. The





recovery pathway is indicated by the dotted line. The previously incurred alkalosis decreased the ventilation, allowing the CO<sub>2</sub> stores to be restocked and simultaneously producing a very low alveolar oxygen concentration. Even at the end of 10 minutes of recovery the R. Q. is still below 0.6 and recovery is far from complete.

Thus hyperventilation and recovery are represented by a continuous cycle which is bisected by the normal R. Q. line.

3. Hypoventilation. An evenly maintained hypoventilation is difficult to achieve in man but it presents the above picture in reverse. We have attempted this by increasing the dead space by breathing through a tube of about 1000 cc. With such a device the total ventilation is enormously increased, but the alveolar ventilation decreased. Figure 5 shows the minute to minute response of three subjects breathing on this device for 10 minutes. As may be seen the original R. Q. was not attained during this period, but as can be predicted from this diagram the alveolar concentrations with further exposures could not have changed very much. The alveolar ventilation shows an immediate reduction from 6.6 to 4.0 liters/minute and then gradually increases and tends to stabilize with an R. Q. of 0.8 and a pCO<sub>2</sub> of 45 mm. Recovery is followed by an immediate rise of alveolar ventilation and high R. Q. indicating that the excess CO<sub>2</sub> stored is now gotten rid off. After 6 minutes of recovery the original equilibrium has been achieved.

Similar pictures have been obtained during intravenous anesthesia by sodium pentathol in man and in dogs. The depression of the respiratory center and the concomitant fall in alveolar ventilation induce the acidosis shift to the left, while recovery from anesthesia is followed by a compensatory high R. Q.

Hypoxia. The effects of hypoxia have been followed in a large number of individuals in the high altitude chamber from ground level to 22,000' simulated altitude (4). Figure 6 shows at 5-minute intervals the average alveolar pathway for 8 men exposed for 30 minutes to 22,000 ft. pressure altitude and the ensuing recovery at ground level. It can be seen that the initial hypoxic ventilation of 12 liters per minute was not maintained. The observed pathway and ventilation are probably a compromise between the hypoxic stimulus and the concomitant inhibition by respiratory alkalosis. Data collected at various altitudes indicate that the normal R. Q. or the steady state is obtained in 30-60 minutes.

CO<sub>2</sub> in the inspired air. When breathing air the inspired oxygen tension is approximately divided up between the alveolar oxygen and the alveolar carbon dioxide, the exact division depending upon the R. Q. and the rate of ventilation. The same concept can be applied to the extra CO<sub>2</sub> added to the inspired air. Since the respiratory center responds to slight changes in the arterial CO<sub>2</sub> tension by high ventilatory rates the pCO<sub>2</sub> is prevented from rising very much. Thus when breathing CO<sub>2</sub> where the inspired oxygen tension is kept as it was before, the gain in alveolar oxygen can be said to equal roughly the inspired CO<sub>2</sub> tension minus the change in alveolar CO<sub>2</sub> tension brought about by this maneuver.

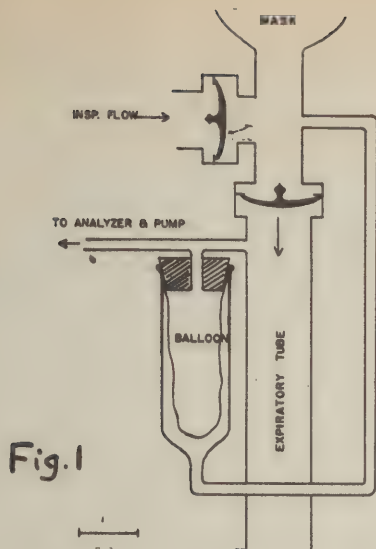
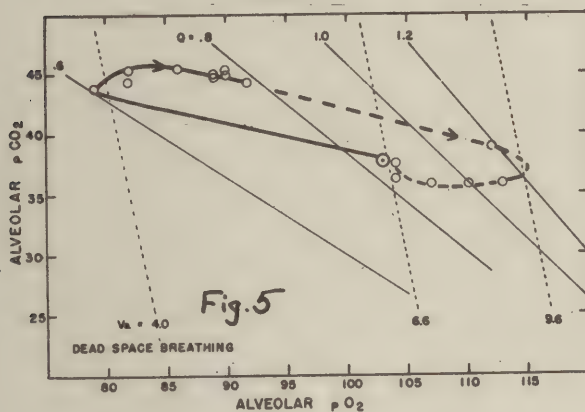
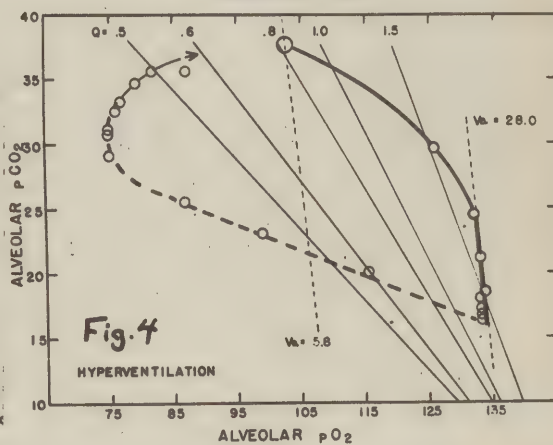
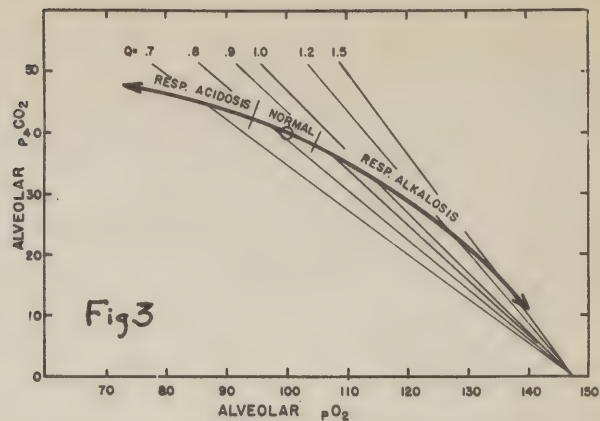
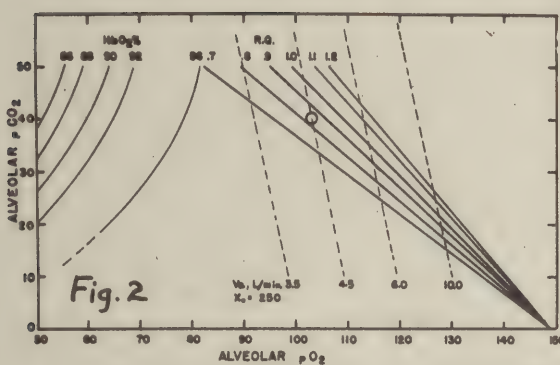


Fig.1





This is well illustrated in figure 7 where the change in alveolar air at five minute intervals is indicated when 22 and 34 mm pCO<sub>2</sub> are inspired at an altitude of 9,500 ft. (Three subjects acclimatized for three weeks at this altitude). The inspired O<sub>2</sub> tension was kept the same with both mixtures. Breathing 22 mm CO<sub>2</sub> the alveolar CO<sub>2</sub> rose 6 mm and the alveolar O<sub>2</sub> rose 18 mm or a total of 24 mm Hg. With 34 mm CO<sub>2</sub> in the inspired air the CO<sub>2</sub> and O<sub>2</sub> changed 11 and 26 mm respectively. Thus at the expense of increased ventilation the alveolar oxygen and oxygen saturation of the hemoglobin was greatly increased with relatively little change in alveolar CO<sub>2</sub>.

Exercise. The fact that the alveolar CO<sub>2</sub> rises during moderate exercise has been appreciated since the work of Haldane. One might predict that under such circumstances the pathway during exercise would simply proceed up the R. Q. line until the new CO<sub>2</sub> level had been reached. However, the minute to minute recordings shown in figure 8 indicate that during the first three to four minutes of exercise (20 cm step, 20 times per minute) there is a relative hypoventilation with its concomitant fall in R. Q. and alveolar oxygen. After this time the steady state is reached with a slightly elevated R. Q. Immediately upon recovery the R. Q. rises precipitously as has been observed by others. However, the interpretation that this is largely due to CO<sub>2</sub> released by lactic acid is not quite tenable. A good share of it must be the metabolic CO<sub>2</sub> retained during the first part of exercise, and which during recovery will be lost in the same way as is the CO<sub>2</sub> accumulation observed with CO<sub>2</sub> breathing, dead space breathing or anesthesia where the lactic acid level is not elevated.

Of further interest is the effect of the inspired oxygen tension upon the exercise pathway. Figure 8 shows the pathway breathing 15, 21 and 100% oxygen. The lower the oxygen concentration the greater the ventilation and the lower is the alveolar CO<sub>2</sub> change. (With 100% oxygen the R. Q. changes cannot be represented on this diagram and any pathway is limited to the R. Q. 1.0 line). This observation suggests that the lower the oxygen tension, the greater the incomplete breakdown products which in turn produce a greater stimulation upon the respiratory system. Preliminary tests on patients with lung diffusion troubles have been tested by Dr. R. Bruce in the Department of Medicine. These people give a response very similar to normal man under low oxygen tension. In fact, in some advanced cases there is practically no change in the alveolar air during exercise.

Thus these work loops might give a quantitative assay of the respiratory efficiency during exercise. The larger the loops, the more efficient the performer.

### Summary

The CO<sub>2</sub>-O<sub>2</sub> diagram offers a new approach to the quantitative description of various respiratory phenomena, since it allows the simultaneous visualization of alveolar oxygen, carbon dioxide, respiratory quotient and ventilation. This approach is made particularly useful

with the continuous recording of alveolar gas composition.

Experiments have been performed which indicate that the alveolar gas concentration can be varied in certain directions only, and along certain pathways. This in a large measure is controlled by the changes in CO<sub>2</sub> output which varies with the relative ventilation. Figure 9 summarizes the principal pathways that have been observed during hyperpnea, hypoxia, hypoventilation, exercise and CO<sub>2</sub> breathing.

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A QUANTITATIVE STUDY OF THE PULMONARY EDEMA  
AND PLEURAL EFFUSION PRODUCED IN RATS BY  
ALPHA-NAPHTHYL THIOUREA AND OTHER THIOUREAS<sup>1</sup>

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Our knowledge of the factors involved in the production and control of pulmonary edema and pleural effusion is still very limited owing in large part to the lack of a simple, reliable method of producing these phenomena experimentally in animals.

Such a method has now become available. From results of recent experiments it is known that several thioureas, particularly phenylthiourea and alpha-naphthyl thiourea (ANTU), produce pulmonary edema and pleural effusion with great constancy in rats and also in a few other animals, dogs, cats and domestic pigs. The facts, a, that rats are readily available and so can be killed and autopsied in adequate numbers at varying intervals after poisoning, and, b, that the degree of pulmonary edema and pleural effusion can be measured (the former by weighing the lungs, the latter by aspirating the fluid into a syringe), should make possible a quantitative attack on the factors involved in the production and control of these two phenomena.

For these experiments 730 domestic and recently trapped wild Norway rats were used. ANTU was administered by stomach tube or intraperitoneal injection in olive oil or acacia. The rats were killed and autopsied, usually at 2-hour intervals over periods of 6 to 60 hours. From 12 to 58 rats were used for each experiment.

The following results were obtained. a. Marked pulmonary edema was often present in less than one hour; pleural effusion was sometimes present at the end of the first hour, but usually did not appear before the end of the 2nd hour.

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<sup>1</sup>Started under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and The Johns Hopkins University and continued under a contract between the Medical Division, Chemical Corps, U. S. Army and The Johns Hopkins University.



b. An inverse relationship appeared to exist between these two phenomena; that is the pulmonary edema decreased as the pleural effusion increased.

c. The maximum level of pleural effusion was about 6 cc per 100 grams body weight; an amount that exactly equals the total blood volume.

d. The amount of pleural effusion did not reach a maximum level until 10 to 12 hours after poisoning.

e. Sub-lethal doses of ANTU sometimes produced maximum amounts of pleural effusion, which indicates that the pleural effusion does not account for the lethal effects.

f. The amounts of the pleural effusion and pulmonary edema varied with age and weight of the animals, the route and vehicle of administration of the drugs and with the available fluids in the body, but not with strain difference.

g. Animals that had been made tolerant to large doses of the drug, no longer showed pleural effusion, but they seemed to show some pulmonary edema.

h. Phenylthiourea and ANTU produced essentially the same amounts of pulmonary edema and pleural effusion.

## RESPIRATORY OBSTRUCTION AND PULMONARY EDEMA

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In the search for the causes of pulmonary edema numerous factors have been implicated under various conditions. Among these factors are relative failure of the left ventricle, permeability changes due to chemical or nerve action, obstruction of the airway, and anoxia. Evidence on the latter two factors is of particular importance to the military physiologist and to the Army Medical Department because of the relationship to the treatment of pulmonary edema.

There are several reasons for believing that lung edema is caused or facilitated by obstruction to the airway, either in the trachea and larynx or in the bronchioles. Thus, in the epidemic of bulbar poliomyelitis in Minnesota in 1946, obstruction by glottic closure or aspiration of secretions was thought to precipitate severe pulmonary edema. It is common clinical experience that edema may occur in the course of a severe attack of asthma. In pulmonary exposure to various types of irritant chemicals, there is evidence that bronchiolar constriction precedes the appearance of edema. The common factor in these circumstances is thought to be the increased negative inspiratory pressure which upsets the normal balance of filtration-colloid osmotic pressure relationships between capillaries and the alveolar space. A "suction-like" action on pulmonary capillaries is postulated. At this juncture certain theoretical considerations should be kept in mind: If one accepts the Starling hypothesis of filtration-reabsorption, the pressures in the lung favor a dry lung. There is a margin of safety of at least 15 cm. water before filtration would exceed reabsorption. Also, the unfavorable pressure relationships in obstructed breathing exist only during inspiration, which is, at most, only half the respiratory cycle. During the remainder of the cycle, conditions are reversed. Furthermore, fluctuations in intrapulmonic pressure are reflected in the thin-walled pulmonary vessels; a large fall in intrathoracic pressure results in a large fall in pulmonary capillary pressure, thereby reducing the filtration gradient.

Previous experimental data on these questions have been inconclusive. Several investigators have demonstrated that high degrees of resistance to inspiration (without expiratory resistance) lead to moderate or severe congestive changes in the lung, but rarely, if ever, to edema. Addition of other factors was necessary to produce edema; thus, Auer and Gates obtained edema in obstructed rabbits by giving adrenalin. Drinker reported early pulmonary edema in a dog which had breathed 10% oxygen against an inspiratory resistance of 9 cm. of water.



On the other hand, Kubichek found no edema when lightly anesthetized dogs breathed against an inspiratory pressure of -30 cm. of water for periods up to 48 hours.

The role of hypoxia in the genesis of edema is likewise not well defined. Although Drinker has shown that inhalation of 10% oxygen leads to increased pulmonary lymph flow and the appearance of red blood cells in the lymph, the procedure did not cause lung edema. Furthermore, the appearance of red cells does not indicate a change in true capillary permeability, since the drug alpha-naphthyl thiourea produces unquestioned changes in capillary permeability, without the appearance of red blood cells in the resulting lymph.

Experiments were designed to add further information on the roles of obstruction and hypoxia in producing edema.

Methods. In the first series of 10 dogs, obstruction was obtained by inserting a tracheal cannula in which the lumen could be reduced to an area of approximately 0.05 sq. cm. This produced a uniformly constricted orifice throughout the respiratory cycle, with resistance varying as a function of rate of air flow.

In a second series of 6 dogs, a fixed inspiratory resistance was obtained by having the animal breathe through a bottle into which air could enter only by displacing a measured column of water. Depth of the tube under water determined the degree of negative inspiratory pressure which then was constant throughout the inspiratory phase. Expiration was unimpeded. In a third series of 4 dogs the procedure was the same as in the second group except that 9% oxygen in nitrogen was substituted for room air as the inspired mixture.

Dogs weighing between 8.5 and 15 kg. were used, with sodium luminal intraperitoneally as the anesthetic (150 mg./kg).

The experimental procedure was to take control observations of respiratory pattern, blood-gas contents, and various pressures, followed by rapid constriction of the airway or introduction of inspiratory resistance to a point which the animal was just able to tolerate. This was usually -15 to -20 cm. H<sub>2</sub>O. Most experiments were terminated at the end of 6 hours. Two of the 20 animals died during the experiment.

In addition to the usual methods of respiration measurement and blood gas analysis, the pneumotachograph was used to record changes in air flow pattern. Low oxygen mixtures were determined by the Pauling Oxygen Analyzer.

The criteria used for the presence of pulmonary edema were the gross and microscopic pathology, lung to body weight ratios, water and chloride estimations in a few animals, and, in the last group, a functional test of the lung diffusion barrier. This test involved the reduction of blood oxygen saturation by inhalation of 9% oxygen, and measurement of the rate of oxygen resaturation when the animal was allowed to breathe room air.

Results. The results are presented in tables 1 and 2, and figure 1. The figure illustrates the characteristics of the air flow curve (pneumotachograph) in the unanesthetized dog, during anesthesia, and during obstruction to both phases of respiration. The latter record demonstrates damping of the respiratory pattern, which is characteristic of any obstruction to breathing. The expiratory phase is greatly prolonged, while inspiration is correspondingly shortened to occupy about 1/3 of the cycle. The resultant changes in air flow velocity effect a much greater increase in inspiratory negative pressure, but the high level is not long maintained. The areas under the inspiratory and expiratory curves are identical.

Table 1 is a partial summary of the respiratory and blood data in dogs obstructed during both phases of respiration. Respiratory minute volume was generally reduced to about 1/3 to 1/2 its control value, with slight decreases in rate. At the end of obstruction there was a pronounced increase in rate and volume, exceeding the control values. In spite of the restricted lung ventilation, arterial oxygen saturation was not appreciably changed except in dog 6 which died at the end of 2 hours. In two animals the level was actually increased. Arterial CO<sub>2</sub> contents were affected, but with trends in both directions in different animals.

The maximum intratracheal pressures represent the mean of a large number of pressure waves. The negative inspiratory pressures were 2 to 3 times the positive expiratory pressures, but of much shorter duration.

Lung/body weight ratios varied from 0.70% to 1.02%, for an average of 0.90% (standard lung/body weight ratio in winter - 0.85% (8)). No edema was found grossly or microscopically, but there was slight to moderate congestion in half the animals.

In the series of dogs listed in table 2, intrapleural pressures measured by a saline manometer varied from -22 to -29 cm. H<sub>2</sub>O, with a return during expiration usually to atmospheric or slightly negative levels. In this series only inspiration was obstructed. The minute volume response was different in this type of obstruction, being unchanged or slightly increased. The respiratory rate, on the other hand, was invariably increased, in some animals to levels of 70 to 90. The rate tended to increase with time, probably indicating the progress of respiratory fatigue.

Arterial oxygen saturation was grossly decreased in 3 animals of this series, to levels of 71, 49, and 62%. Carbon dioxide content, however, tended to decrease rather than increase, in amounts commensurate with the increase in pulmonary ventilation.

Lung/body weight ratios were almost identical to those in the first series (average .875%). Pathologically there was pleural transudation in one animal, a total of 30 cc. of blood-tinged fluid. Grossly and microscopically there was slight to moderate congestion in all animals with some areas of extensive alveolar hemorrhages microscopically.



In the group of animals breathing 9% O<sub>2</sub> in combination with inspiratory obstruction, most data paralleled observations on the group without hypoxia. Arterial saturations of 34 to 65% invariably returned to control levels after 2 minutes of air breathing, indicating no significant lung-blood diffusion barrier. The lungs at post mortem looked surprisingly healthy.

Summary. Severe tracheal obstruction has been produced in normal dogs, during both phases of respiration and during inspiration alone. In spite of marked changes in pulmonary ventilation and alteration in pulmonary dynamics, no pulmonary edema has been obtained. More severe congestive changes are caused when obstruction is confined to inspiration than when operating in both phases of the cycle.

In obstruction to both phases, the negative pressures in inspiration are to a very large extent counterbalanced by the increased positive pressures in expiration.

While changes in intrapulmonary and intrathoracic pressures of the range tested may contribute to the production of pulmonary edema, by themselves they do not produce edema in the normal dog.

Hypoxia of a degree usually considered causative of pulmonary edema produced no edema, even in combination with inspiratory obstruction.



Fig. 1. Air flow and intratracheal pressure curves during anesthesia and obstructed breathing.



Table 1. Respiratory Effects of Fixed Tracheal Obstruction

Dog No.	Duration Obstruction	Reduction in Ventilation	Average Peak Intratracheal Pressure		Arterial O <sub>2</sub> Saturation		Arterial CO <sub>2</sub> Content	Lung/Body Autopsy Wt. Ratio	Remarks
			Hours	% of Control	Cm. H <sub>2</sub> O	%			
GROUP 1									
3	0								
	4	41			-10.7	83.1	43.3		
	6	50				93.1	47.2		
4	0								No edema or
	3	69			-15.7	85.9	48.8		
	6	47				83.8	42.7	1.01	congestion
5	0								No edema or
	3	42			-14.5	74.8	48.1		
	6	60				94.6	45.5	0.99	congestion
6	0								Moderate
	2	28			-15.0	79.8	51.3	0.84	congestion
7	0								No congestion
	4	59			-15.2	88.1	42.4	0.92	or edema
8	0								Slight
	3	56			-11.8	86.7	43.0		congestion -
	5	56				83.3	42.7	1.02	no edema
9	0								Slight
	3	40			-9.0	87.5	46.9		congestion -
	6	35				87.7	48.0	0.84	no edema
10	0								
	3	63			-12.2	88.3	42.7		
	6	84				85.6	41.8		
						83.3	39.4	0.70	

Table 2. Respiratory Effects of Increased Inspiratory Negative Pressure

Dog No.	Duration Obstruction	Maximum Inspiratory Pressure		Arterial O <sub>2</sub> Saturation	Arterial CO <sub>2</sub> Content	Lung/Body Wt. Ratio	Lung Pathology
		Hours	Cm. H <sub>2</sub> O				
GROUP 2							
12	0			90.3	41.9		Slight congestion
	4	-23		85.3	42.4	0.90	
13	0						Slight congestion
	1	-26		89.8	36.2		
14	0			90.4	34.0	0.80	Moderate congestion
	4	-25		84.0	48.6	0.80	
15	0			88.6			Slight congestion
	4	-26		84.7		0.96	
16	0			92.5	44.8		Moderate congestion
	4	-28		61.8	31.3	0.76	
GROUP 3 (9% O <sub>2</sub> )							
Intratracheal							
26	0			96.3 <sup>1</sup>	42.8		Slight congestion
	3	-24		82.7 <sup>1</sup>			
	6			84.7 <sup>1</sup>	32.7	0.69	
28	0			81.5	49.1		Slight congestion
	3	-21					
	6			90.5 <sup>1</sup>	32.3	0.94	
29	0			95.5	45.9		Slight congestion
	3	-20				0.85	
	6			92.5 <sup>1</sup>	30.6		
30	0			83.7	46.5		Slight congestion; many hemorrhages; (distemper)
	3	-15				1.1	
	6			83.6 <sup>1</sup>	33.5		

<sup>1</sup>Values obtained after 2 minutes breathing air.





## NORMAL HUMAN ARTERIAL OXYGEN SATURATION;

### ITS RATE OF INCREASE UPON BREATHING 100% OXYGEN

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Until recently, normal human arterial oxygen saturation was considered to be 93 to 97%. Roughton analyzed critically certain errors inherent in the measurement of  $O_2$  capacity by the Van Slyke manometric technique and concluded that this figure should be revised upward to 97%. At about the same time, Drabkin and Schmidt using a spectrophotometric technique, arrived at a figure of 98 to 99% for arterial oxygen saturation in normal men breathing room air. We have obtained further information upon this point by measuring (a) the increase in oxygen content of arterial blood, and (b) the increase in oximeter saturation when twenty normal men previously breathing room air inhaled 100%  $O_2$ . Data obtained from (a) indicate that normal saturation during inhalation of room air is 97.5% (average) while the oximeter data (b) indicate a normal saturation of 96.2%. It is possible that metabolism of the ear tissues can explain the discrepancy.

We have noted that the rate at which arterial  $O_2$  saturation, measured by the oximeter, rises following the inhalation of 100%  $O_2$  is considerably slower than expected. This oximeter rise was measured in 45 healthy white male subjects. The subjects were sitting quietly and the oximeter saturation reading was arbitrarily set at 95 to 96% while the subjects were breathing room air. They were then given pure  $O_2$  to breathe and oximeter readings were made at 10 second intervals for two minutes, and at 30 second intervals thereafter until 5 minutes had elapsed. After an average delay of 7.1 seconds following the first inhalation of  $O_2$ , the oximeter beam began to move. This delay, representing total mouth to lung to ear time, may prove useful as an objective index of pulmonary circulation time. Following this lag, the saturation rose quickly and then approached the maximum more slowly. This maximum was reached in a mean time of 132 seconds and thereafter remained relatively stable for the duration of the 5 minutes. Because of minor instrumental and physiologic fluctuations about this maximum, we used the time to maximum minus 0.5% as the index of complete saturation. This level was reached in a mean time of 52 seconds.

It is customarily held that hemoglobin is completely saturated with  $O_2$  at an arterial  $pO_2$  of 159 mm. This tension, 160 mm., is reached in the lung air within 1 to 3 seconds. If this time be added to the lung to ear circulation time lag of 7 seconds, and the instrumental lag to complete deflection of 5 to 6 seconds, the total delay



is only 15 seconds, after which one might expect complete arterial saturation to be reached. However, there is a delay of at least 30 to 35 seconds between the expected time of about 15 seconds and the recorded time of 52 seconds.

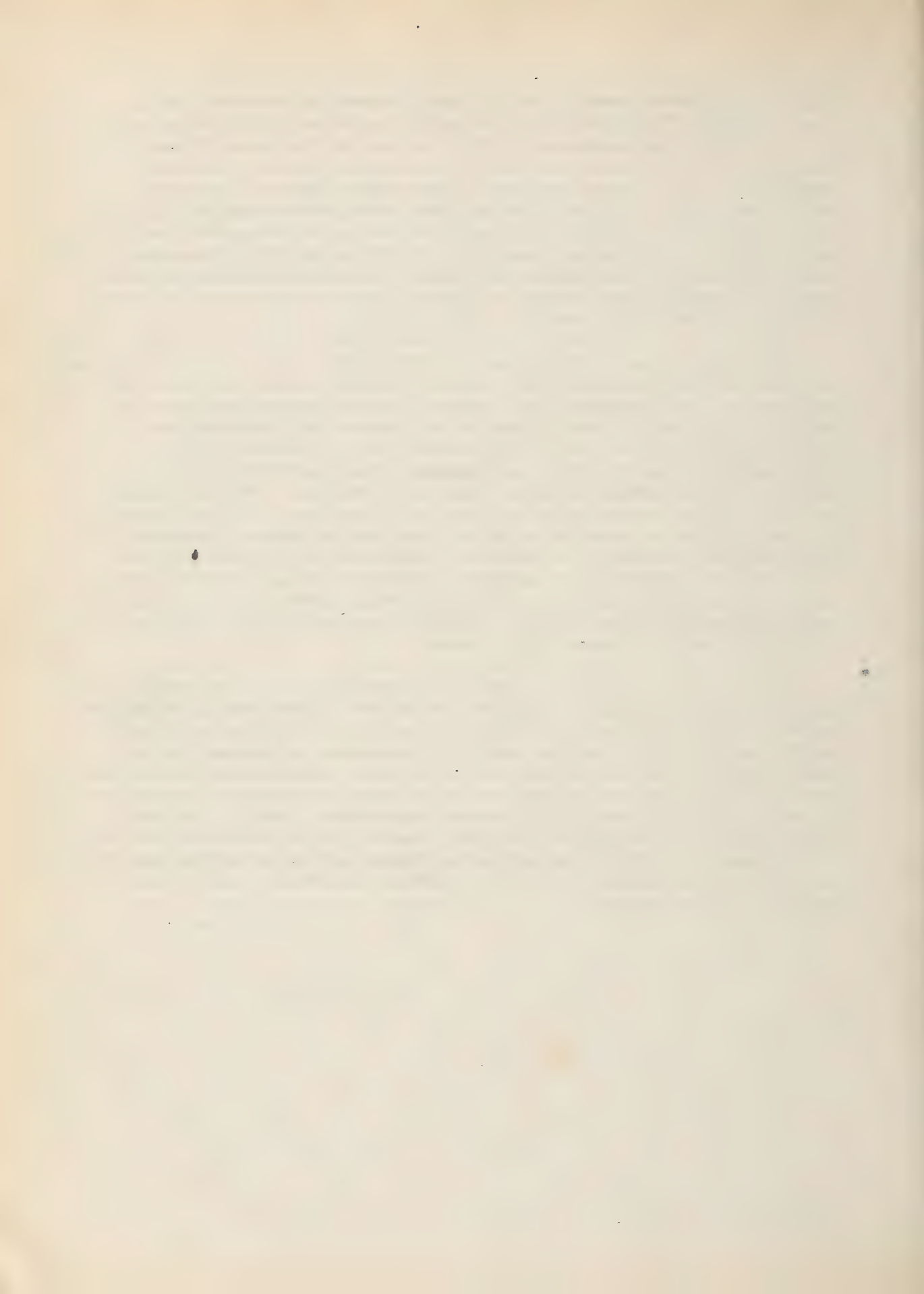
Various explanations may be proposed for this delay. The simultaneous use of the oximeter and the nitrogen meter (for continuous analysis of the nitrogen content of respired gases) has permitted us to investigate these explanations. The mean nitrogen content of the gas expired at the end of successive expirations has been measured during the first two minutes of O<sub>2</sub> inhalation. The mean N<sub>2</sub> content of the terminal expired air after one breath of oxygen was 65% (S.D.  $\pm$  7.3%). Assuming an alveolar CO<sub>2</sub> of 5.6%, this represents an oxygen tension of about 210 mm Hg. However, this value, representing the gas expelled at the end of a normal expiration, well after the dead space has been cleared, does not truly represent the "deeper" alveolar gas. In the same 42 subjects, after quiet expiration of the second breath of oxygen, a further maximal forced expiration was made. The nitrogen content of this "deeper" alveolar air had a mean value 3.9% greater than the value of the gas expired at the end of the normal quiet expiration. This finding demonstrates the non-uniformity of alveolar air. It is possible that non-uniformity represents differences of ventilation in various regions of the lung, and delayed ventilation of some alveoli through which blood is circulating. Experiments with hyperventilation were made to test this possibility. Hyperventilation caused a much faster oximeter rise. One might conclude that hypoventilation existed during quiet breathing and was responsible for the slow oximeter rise, and that voluntary hyperventilation had reduced the hypoventilation of certain areas of the lung. However, vigorous breathing may not only facilitate ventilation of poorly aerated parts of the lung, but also increases the pO<sub>2</sub> more rapidly in the well ventilated areas. We attempted to differentiate these two factors by the inhalation of 40% O<sub>2</sub>. 40% O<sub>2</sub> was chosen since its inhalation should result in an alveolar pO<sub>2</sub> at equilibrium of about 240 mm. Hg (well in excess of 159 mm. Hg). Seven subjects breathed 40% O<sub>2</sub> for 5 minutes, which should be sufficiently long to reach equilibrium and overcome any delay due to hypoventilation. The oximeter rose a mean of 2.43% and became stable. Then 100% O<sub>2</sub> was given. In all cases a further increase in saturation occurred within 1 minute. The mean increase was 0.89%; the standard error of the mean was  $\pm$  0.091, giving a "t" value of 9.9, indicating the increase to be a highly significant variation from zero. Further attempts to overcome delayed ventilation were made by breathing 40% O<sub>2</sub> for 5 minutes, then voluntarily hyperventilating for 30 seconds, followed by another two minutes of quiet breathing. Then again 100% O<sub>2</sub> was given, and the saturation rose a mean of 0.82%. In other words, attainment of the last 1% of arterial saturation, which accounts for much of the delay we are trying to explain, appears to be a function of very high alveolar and arterial oxygen tensions, and not of hypoventilation of certain areas of the lung. This suggests that the customary belief that a pO<sub>2</sub> of 159 mm. Hg will completely saturate arterial blood is wrong, and that much higher tensions are required. However before this conclusion can be accepted, one must answer certain questions. First, are there shunts through or around the lungs delivering enough venous blood into the

arterial circulation so that even the excess O<sub>2</sub> dissolved in the blood equilibrated with 40% O<sub>2</sub> is insufficient to saturate it? The findings of Berggren and of Fasciolo and Chiodi that the arterial pO<sub>2</sub> of persons breathing 100% O<sub>2</sub> is only 10 to 30 mm. below the alveolar pO<sub>2</sub> eliminates the shunt explanation. Second, is the metabolism of the ear contributing some unsaturation to the blood we are measuring with the oximeter, and is this overcome only by very high arterial oxygen tensions? For various reasons we do not believe this factor is responsible. Third, is it a valid assumption that the alveolar to arterial pO<sub>2</sub> gradient remains small during the period of rapid change in alveolar pO<sub>2</sub>? Preliminary studies suggest that this assumption is valid.

The extreme upper end of the O<sub>2</sub> dissociation curve is not well defined, due to technical limitations. However, it is believed that oxygenation of Hb proceeds in 4 steps. We may be dealing here with only the last one of these steps, and there is reason to believe that very high O<sub>2</sub> tensions should be required for achieving the last 1% of saturation. Moreover the proposed relationship would be a reciprocal relationship between reduced Hb and pO<sub>2</sub>. Both the shape of the oximeter curves on breathing O<sub>2</sub> and the shape of the descending oximeter curve when breathing of room air is resumed, suggest that they are following a reciprocal relationship. Therefore, the slow rise of arterial O<sub>2</sub> saturation becomes reasonable and predictable on theoretical grounds. The factors of venous-arterial shunts, pulmonary hypoventilation and ear metabolism are undoubtedly present, but are probably of lesser importance.

The rate of increase of arterial saturation is the end result of various processes, such as a, movement of O<sub>2</sub> to the alveoli, which involves mixing of gases in the lung; b, diffusion of O<sub>2</sub> across the alveolar membrane into the red cell; c, conversion of reduced Hb to HbO<sub>2</sub>; and d, transport of HbO<sub>2</sub> to the site of measurement. As these various lung functions are disturbed by disease, one might expect the disturbance to be revealed in such a measurement. From a study of this test in patients with pulmonary disease, we believe that the rate of increase in arterial saturation on breathing oxygen may be useful as a simple, innocuous, objective, overall measurement of certain aspects of lung function.





# PHOTOELECTRIC DETERMINATION OF ARTERIAL

## OXYGEN SATURATION IN MAN

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The Millikan and Goldie oximeters (1,2) have greatly facilitated the study of changes in arterial oxygen saturation in man. However, since these instruments must be preset to known arterial saturation values they cannot be conveniently used on patients who may have arterial hypoxia, nor can they be used for the actual determination of arterial oxygen saturation. Furthermore, extensive calibration studies with the Millikan compensated circuit oximeter carried out in this laboratory have yielded such variable results (3) that it has been concluded that oximeter saturation readings are only of qualitative value in indicating changes in arterial oxygen saturation. Consequently studies have been carried out in an attempt to eliminate or minimize these shortcomings of the instrument.

A device is described herein which can be used to measure the absolute value of and follow continuously the arterial oxygen saturation from a pickup unit attached to the pinna of the human ear. This is accomplished by means of a photoelectric earpiece which allows simultaneous measurement of the transmission of red and near infra-red light through either the normal heat flushed or the bloodless ear. Then by calculation, the light transmission of the blood alone in these spectral regions can be determined and in turn the percentage of oxygen saturation of this blood derived.

Instrument Operation and Construction. Although it was originally assumed that the Millikan oximeter responded to transmitted light in the region of the red and the green (1) it has been pointed out that the ear transmits practically no light of a wave length shorter than 600 millimicrons (4). Therefore the operation of the oximeter is based on differences in transmission of light by oxygenated and reduced hemoglobin which occur in the red and near infra-red rather than in the red and green portions of the spectrum as originally supposed.

The Millikan oximeter earpiece contains two barrier layer iron-selenium photronic cells each covered by a specific Wratten gelatin optical filter. Assuming a constant earpiece light source the amount of light transmitted through the pinna of the ear and filters to the photoelectric cells is a function of the fixed characteristics of the

respective filters, the amount of ear tissue and the amount and state of oxygenation of the blood in the path of the light.

One of the photocells is covered by a green Wratten 61N filter which transmits light in the spectral region from 480 to 600 millimicrons and in the region above 750 millimicrons. This cell which has been called the "green cell" actually responds to light in the near infra-red. This is due to the fact that the ear absorbs practically all light of wave length shorter than 600 millimicrons and the photocell response falls practically to zero for light of wave length greater than 800 millimicrons. Therefore this cell (the "infra-red cell") is activated only by light of a wave length of approximately 800 millimicrons. Since oxygenated and reduced hemoglobin transmit light of this wave length to practically the same degree (5) the output of the infra-red cell is a function of the amount of ear tissue and blood in the optical path and is independent of the degree of oxygenation of this blood.

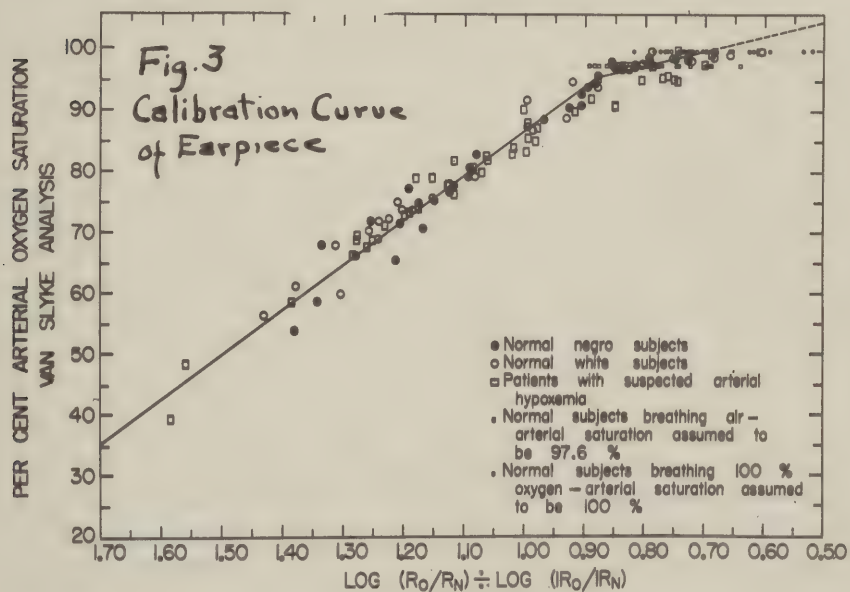
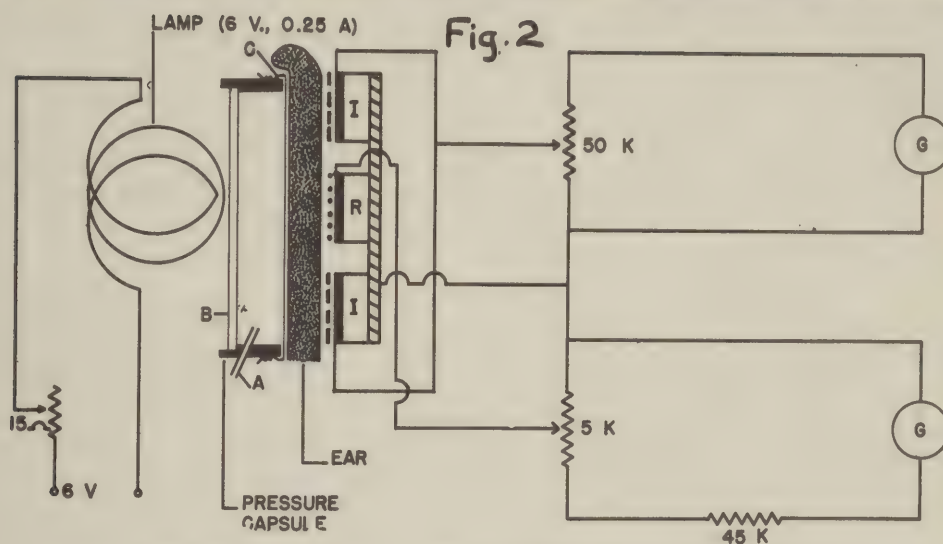
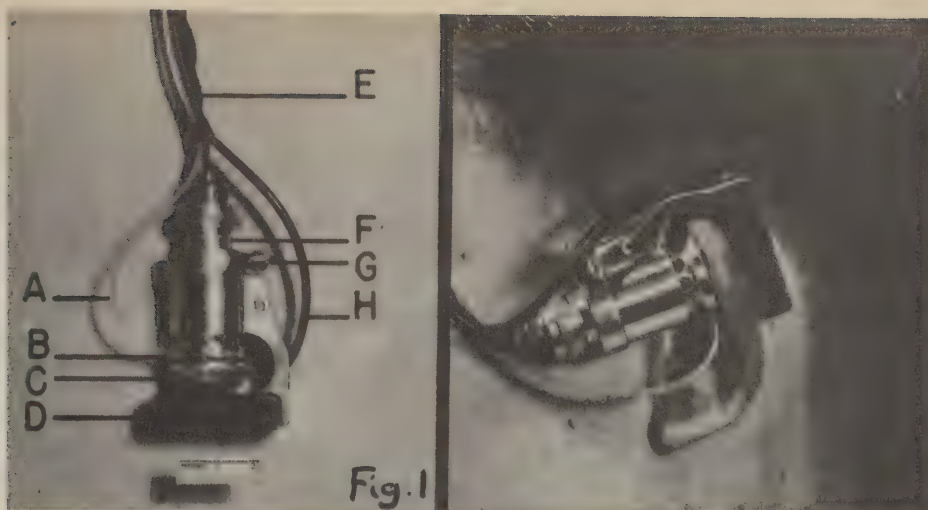
The other photocell is covered by a red Wratten 29F filter which transmits light of wave lengths above 600 millimicrons. The percentage transmission of red light (600 to 750 millimicrons) is very different for oxygenated and reduced hemoglobin (6). Hence the output of this cell (the "red cell") is a function of the amount of ear tissue and both the amount and the degree of oxygenation of the blood in the path of the light. If the ear tissue and amount of blood are constant the output of the red cell is a function of the oxygen saturation of the blood interposed in the optical path of the earpiece (6, 7).

In the Millikan oximeter the amount of ear tissue in the optical path is corrected for by adjusting the instrument to indicate a known value of saturation (usually 98 or 100% when the subject is breathing air or 100% oxygen) while the earpiece is in place on the ear on which it is to be used. The amount of blood in the optical path is compensated for automatically by bucking the output of the infra-red cell against the output of the red cell.

The instrument to be described is based on the same spectral transmission characteristics of hemoglobin as is the Millikan oximeter. It differs, however, in the type of earpiece, infra-red filter and electrical circuits used.

The earpiece (fig. 1) differs from the Millikan oximeter earpiece in that the light source instead of being fixed in position is incorporated into an adjustable cylindrical housing designed so that it can be fixed securely in the desired position relative to the ear. The end of the light housing which comes in contact with the ear contains an air-tight chamber, the pressure capsule. The surface of the pressure capsule towards the light bulb is of glass while the surface towards the ear is covered with thin translucent rubber dam; thus absorption by the capsule of the light being transmitted from the light bulb to the ear is relatively small. A 2-centimeter length of No. 22 gauge hypodermic tubing serves as an air inlet to the pressure capsule





and is connected to a hand bulb and mercury manometer by means of a suitable length of polythene tubing. When the earpiece is placed on the ear the unit housing the light is adjusted so that the end (covered with rubber dam) fits snugly but not tightly against the front surface of the pinna. Thus when the pressure capsule is inflated to arterial occlusive pressures (200 mm. of mercury) the pinna is compressed between the rubber diaphragm and the flat, front surface of the photocell housing so that the blood is squeezed from the portion of the ear within the optical pathway of the earpiece. The photocells are covered with a mask containing a circular opening 12 millimeters in diameter which is 6 millimeters less than the diameter of the surface of the pressure capsule. Masking of the photocells in this manner assures that all light reaching the cells is transmitted through a portion of the ear which can be efficiently pressurized by the capsule.

An infra-red Wratten No. 87 gelatin filter is used to cover the infra-red cell in place of the Wratten No. 61N filter in the ordinary oximeter earpiece because it was found that the "bloodless ear" transmits significant amounts of light in the region of the green.

The outputs of the red and the infra-red cells are recorded separately by means of galvanometers\* which have a sensitivity such that 0.006 microamperes produces a deflection of 1.0 millimeters. A schematic diagram of the circuit used is shown in figure 2.

Procedure. The earpiece is placed on an optical filter\*\* (E-100) and allowed to become warm. The infra-red and red cell outputs are adjusted by regulating their respective variable resistors to produce equal galvanometer deflections (usually 50 to 70 mm. depending upon the anticipated optical density of the ear). The earpiece is then placed upon the ear and readings are taken at 60 second intervals until a constant value is reached, indicating that the ear has become fully flushed. Usually this occurs within 10 minutes. The pressure capsule is then inflated to 200 mm. of mercury and readings are taken at 30 second intervals for a period of 5 minutes. The pressure is then released and readings are taken for an additional 5 minute period

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\*Rubicon box galvanometers (catalogue No. 3415) with a coil resistance of 1500 ohms, a critical damping resistance of 40,000 ohms and a period of 5.7 seconds were used. A single galvanometer can be used by incorporating a double pole double throw switch so that either the red or infra-red circuit can be connected individually to the single galvanometer.

\*\*The E-100 filter is composed of the following materials in the order in which they are named: 1. One piece ground glass 2.09 mm. in thickness. 2. One layer of tracing paper (albanene tracing paper, 195L-12 Keuffel and Esser Company, Hoboken, N. J.). 3. One Wratten 29F filter. 4. Three layers of tracing paper. 5. One Wratten 29F filter. 6. One layer of tracing paper. 7. One plain glass slide measuring 1.14 mm. in thickness.



or until the completion of some experimental procedure. Readings are usually taken at 10 to 30 second intervals during withdrawal of arterial blood samples and/or administration of low oxygen mixtures. If the experimental procedure is of more than 30 minutes duration the "bloodless ear readings" (pressure capsule inflated to 200 mm. of mercury) are redetermined at the end of the experiment.

Calculations. The amount of light absorbed by the portion of the ear interposed between the light source and photocells of the earpiece is inversely proportional to the logarithm of the galvanometer deflection produced by the output of the photocells. Furthermore, the total light absorption by this portion of the ear is an additive effect of the light absorbed by the ear tissue proper and by the blood contained in this ear tissue. Consequently the amount of light absorption by the blood contained in this ear tissue is a function of the difference between the light absorption of the bloodless ear and the light absorption of the blood containing ear.

Therefore, a value which is a function of the absorption of infra-red light by the blood ( $IR_B$ ) can be obtained by the following equation:

$$IR_B = \log IR_O - \log IR_N = \log \frac{IR_O}{IR_N}$$

in which  $IR_O$  is the galvanometer deflection produced by the infra-red cell when the ear is rendered bloodless by the pressure capsule and  $IR_N$  is the galvanometer deflection produced by the infra-red cell when the ear is in the normal blood containing state.

Similarly, a value ( $R_B$ ) which is a function of the absorption of red light by the blood interposed in the optical path of the earpiece can be obtained by the expression  $R_B = \log \frac{R_O}{R_N}$  in which  $R_O$  is

the galvanometer deflection produced by the red cell when the ear is rendered bloodless and  $R_N$  is the galvanometer deflection obtained from the blood containing ear.

The light absorption by blood in the near infra-red is a function of the total amount of hemoglobin contained in this blood. Likewise, absorption of red light by this blood is a function of the amount of oxyhemoglobin. Therefore, the ratio  $R_B/IR_B$  is a function of the oxygen saturation of the blood interposed in the optical path of the earpiece. Since this blood is rendered arterial by the increase in blood flow through the ear produced by the heat of the earpiece, the expression  $R_B/IR_B$  gives a value which is a function of the percentage saturation of arterial blood with oxygen.

The exact nature of the relationship between  $R_B/IR_B$  and arterial oxygen saturation may be determined experimentally and a calibration curve which will allow oxygen saturation to be determined from  $R_B/IR_B$  ratios may be constructed.



## Methods

The calibration and range of error of the instrument has been studied in three ways: (1) The ratio  $R_B/IR_B$  has been calculated from the readings of the instrument obtained when recording from 35 ears of 23 normal subjects (age range 5 to 68 years) while breathing room air and 100% oxygen. The values of  $R_B/IR_B$  obtained for each ear have been plotted against the arterial oxygen saturation assuming that the arterial saturation of these subjects was 97.6\* and 100% when breathing air and oxygen respectively. The average values obtained have been used to establish the calibration curve of the instrument in the region from 98 to 100% arterial oxygen saturation (fig. 3). (2) Utilizing an indwelling arterial needle blood samples have been obtained from the radial artery of 5 normal white and 5 normal negro subjects after periods of two and one half to ten minutes breathing each of the following seven gas mixtures: air, 100% oxygen, and 16, 14, 12, 10 and 8% oxygen in nitrogen. Instrumental readings were recorded at 10 second intervals throughout the period of withdrawal of each of 62 arterial blood samples. These readings were averaged and the average value of  $R_B/IR_B$  for each sampling period was calculated. The values obtained have been plotted against the arterial oxygen saturation as determined by Van Slyke analyses (8) of the simultaneously withdrawn arterial blood samples (fig. 3). (3) Similar methods have been used to obtain instrumental readings during withdrawal of 48 arterial blood samples from a series of 19 patients with suspected arterial hypoxemia. Arterial hypoxia in these cases was a result either of congenital cardiac anomalies or of acquired respiratory abnormalities. Polycythemia was present in the majority. The average values of  $R_B/IR_B$  calculated from the instrumental readings obtained over the period of arterial sampling have been plotted against the arterial oxygen saturations determined by the Van Slyke analysis of the blood (fig. 3).

## Results

The plot of the calculated values of  $R_B/IR_B$  against the simultaneous estimations of arterial oxygen saturation by Van Slyke analysis (fig. 3) does not reveal significant differences in the data obtained from the three groups of subjects studied. Therefore, all of these data have been used as a basis for constructing a calibration curve for the instrument. At levels of arterial saturation below 95% the relationship between  $R_B/IR_B$  and the arterial oxygen saturation is apparently linear (fig. 3). The linear regression line has been calculated for all values of  $R_B/IR_B$  at arterial saturation below 95% and the resulting line has been taken as the calibration of the instrument in this region of arterial saturation. The average values of  $R_B/IR_B$  obtained from normal subjects breathing air and oxygen have been plotted at 97.6 and 100% arterial saturation respectively. The 97.6 and 100% points obtained in

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\*Average value based on Van Slyke analyses of arterial blood samples obtained from 29 resting subjects breathing air (altitude 1100 feet). Extreme values: 94.1 - 100%.

this manner have been connected by a straight line. The calibration curve of the instrument was completed by extending these two straight lines to their point of intersection (fig.3).

Utilizing these calibration lines\* the photoelectrically determined values of arterial oxygen saturation of 20 normal subjects when breathing 100% oxygen ranged from 97.1% to 104.2%. These subjects ranged in age from 5 to 68 years. Six were negroes (table 1). The average arterial oxygen saturation as determined by Van Slyke analyses of 61 arterial samples obtained from 24 normal subjects while breathing 100% oxygen was 99.0% and the range of values was from 96.1 to 101.1%.

The photoelectric determinations of arterial oxygen saturation of the 23 subjects when breathing air ranged from 94.9 to 101.2% (table 1). The average arterial oxygen saturation as determined by Van Slyke analyses of 57 arterial samples obtained from 29 normal subjects while breathing air (altitude 1100 feet) was 97.6%. The range of values was from 94.0 to 100.0%.

At values of arterial saturation between 40 and 95%, the average difference between simultaneous photoelectric and Van Slyke determination of arterial oxygen saturation was 0.21 percentage saturation points. The range of the difference was from -5.6 to 5.9 percentage points. Ninety per cent of the differences were less than 5 percentage saturation points and 75% of the differences were less than 3 percentage points. The range of variation was not appreciably different for white and negro subjects and for patients with variable degrees of polycythemia (table 1).

### Discussion

Photoelectric determination of the arterial oxygen saturation of blood samples in vitro has been successfully carried out by several investigators (7,9,10-15). Photoelectric determination of changes in arterial oxygen saturation has also been accomplished in unopened arteries and veins and in the intact pinna of the human ear (7,1,2).

The present report represents, to the authors knowledge, the first description of the determination of absolute values of arterial oxygen saturation in the intact pinna of the human ear. Squire (16) in 1940 described a method for the determination of the quantity of

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\*The applicability of this calibration curve to different earpieces has not been determined. It is of interest that the instrument predicted the arterial saturation satisfactorily: (1) when used on a patient whose blood contained  $3/4$  grams of sulfhemoglobin per 100 cc., (2) when used over a scabbed and blistered area on the ear, and (3) when used on the ear, a fold of skin on the neck and a fold of skin on the chest of a patient with cutis laxa.



blood in the web of the hand and its degree of oxygenation. He did not, however, use the method to determine arterial oxygen saturation and presents no data as to the accuracy of the method for determining the degree of oxygen saturation of the blood contained in the web of the hand.

The accuracy of the method described herein appears adequate for intermittent or continuous clinical determination of arterial oxygen saturation and should obviate the necessity of arterial punctures for this purpose. The accuracy of the instrument in use at the present time is in part limited by the accuracy of the galvanometer readings. The instrument must be set so that the galvanometer deflections obtained with the "bloodless ear" are on scale (i.e., 120 mm. with the galvanometers used). When the instrument is adjusted in this manner the average red cell deflection when subjects were 100% saturated was 60 mm. and was 40 mm. when these subjects' saturation was decreased to an average value of 62% by breathing 8% oxygen. Under these circumstances an error of 1 mm. in reading the galvanometer deflection produced by the red cell produces an error of approximately 3% in the predicted value of arterial saturation.\*

The accuracy of the instrument was not disturbed by walking on a treadmill at 1.7 miles per hour; consequently it would appear to be readily applicable to the study of arterial oxygen saturation during various cardiovascular-respiratory function tests and during operative procedures.

Since the instrument was found to perform satisfactorily on very deeply pigmented ears (negroes) it is probable that the method can be applied to the determination of oxygen saturation of the blood contained in any human or animal tissue which will allow application of a pickup unit and an incorporated pressure capsule so that the interposed tissue can be successfully transilluminated and subjected to arterial occlusive pressures.

### Summary

An instrument is described for the photoelectric determination of absolute values of arterial oxygen saturation in the intact pinna of the human ear.

The instrument has been calibrated by comparing the readings obtained with arterial oxygen values determined by Van Slyke analysis of 108 simultaneously withdrawn arterial blood samples.

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\*Recently the mirror arrangement in the galvanometer has been modified so that a full scale deflection of 360 mm. is obtainable. Thus the error in saturation produced by the error in reading the galvanometer has been reduced to less than 1%.



In the range from 40 to 95% saturation of arterial blood with oxygen the average difference between photoelectric and Van Slyke determination of arterial oxygen saturation was 0.21 percentage saturation points. The range of difference was -5.9 to 5.6\*\* percentage points and the standard deviation 2.4 percentage points.

The accuracy of the instrument does not appear to be affected by age, race, sex or blood hemoglobin content of the subjects on which it is used.

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\*\*Recently photoelectric readings were obtained, during periods of breathing air and 100% oxygen, simultaneously with withdrawal of two arterial blood samples from a markedly cyanotic three year old boy with congenital heart disease. Van Slyke analysis of these samples yielded oxygen saturation values of 13.4 and 14.3% respectively. On the basis of the extrapolated calibration curve the respective photoelectric estimations of arterial saturation were 22.1 and 24.8%. This is the lowest value of arterial saturation ever obtained on this laboratory.

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Table 1.

AVERAGE<sup>1</sup> CALIBRATION DATA OF PHOTOELECTRIC EARPIECE FOR DETERMINATION  
OF ABSOLUTE VALUES OF PERCENTAGE SATURATION OF ARTERIAL BLOOD WITH OXYGEN

	No. of arterial samples	$\frac{\text{Log } (R_o/R_N)}{\text{Log } (I_{R_o}/I_{R_N})}$	% O <sub>2</sub> Saturation Arterial Blood Photoelectric estimation on ear	Van Slyke analysis of blood	Difference: percentage saturation points (A - B)	Oxygen capacity cc. O <sub>2</sub> per 100 cc. art. blood
20 Normal subj. <sup>2</sup> 100% O <sub>2</sub>	9	0.700 (0.52-0.82)	100.0 (97.1-104.2)	99.7 (97.3-100)	0.3 (-0.2-1.6)	19.2 (17.8-22.9)
23 Normal subj. <sup>3</sup> air	10	0.800 (0.64-0.89)	97.6 (94.8-101.4)	97.5 (94.1-100)	0.1 (-1.7-1.9)	19.1 (17.8-22.4)
10 Normal subj. 16% O <sub>2</sub>	10	0.906 (0.83-1.00)	93.6 (87.4-97.5)	92.4 (87.6-97.2)	1.2 (-0.2-1.6)	19.2 (17.8-22.4)
5 Normal subj. 14% O <sub>2</sub>	8	1.092 (0.99-1.20)	80.4 (72.2-88.1)	80.8 (73.9-87.0)	-0.4 (-1.8-1.1)	19.1 (18.4-20.3)
9 Normal subj. 12% O <sub>2</sub>	9	1.117 (0.92-1.26)	78.6 (68.3-92.9)	79.7 (70.4-95.0)	-1.1 (-4.6-1.7)	19.1 (17.7-22.5)
9 Normal subj. 10% O <sub>2</sub>	9	1.210 (1.12-1.28)	71.8 (66.7-78.5)	72.4 (66.3-77.8)	-0.6 (-4.6-4.0)	19.3 (17.8-22.4)
10 Normal subj 8% O <sub>2</sub>	10	1.340 (1.21-1.45)	62.5 (55.1-71.5)	62.2 (53.6-69.1)	0.3 (-5.6-5.9)	19.1 (17.8-22.4)
19 Patients arterial hypoxemia	48 <sup>4</sup>	1.074 (0.61-1.89)	80.2 (22.1-102.4)	79.1 (13.4-102.5)	1.1 (-5.1-10.5)	(16.4-35.7)

<sup>1</sup>Figures in parenthesis are extreme values.

<sup>2</sup>Determinations were made on both ears of 9 of these subjects making a total of 29 ears studied while subjects were breathing 100% O<sub>2</sub>. Average age of subjects was 30 years (3-68); six of the subjects were negroes.

<sup>3</sup>Determinations were made on both ears of 12 of these subjects.

<sup>4</sup>Arterial samples obtained under various conditions including: rest, walking on a treadmill, and breathing gas mixtures containing from 10 to 100% O<sub>2</sub>. Average age of patients, 27 years (3-55).



# THE EFFECT OF MECHANICAL VIBRATION ON THE KNEE JERK OF THE CAT

D. E. Goldman, Lt. Comdr., HS, USNR

In 1938, Coermann (1) surveyed some of the physiological effects of the exposure of man to mechanical vibration in the frequency range 20 to 1000 cps. One of the few positive results obtained was that during the operation of a vibrating platform on which a human subject was seated, it was difficult or impossible to obtain the patellar reflex. This phenomenon was further investigated by Loeckle (2). Briefly, he found that application of a vibrating rod to the skin area over the course of the femoral artery, vein and nerve produced immediate abolition of the reflex, whereas if the rod was applied directly to the muscle there was no effect. Further, in one experiment on an anesthetized cat, he found that application of the vibrator to the femoral artery, which had been lifted free from the surrounding tissues for a few centimeters distance, abolished the reflex. If the artery was stripped of its adventitial layer, the inhibitory effect vanished. In addition, he studied an individual on whom a unilateral lumbar sympathectomy had been performed one year before. On the sympathectomized side only, it was impossible to inhibit the patellar reflex by vibration. He used frequencies from 30 to 100 cps. and amplitudes up to one millimeter. Loeckle naturally concluded that the inhibition was mediated by the sympathetic fibers of the periarterial plexus and the sympathetic ganglionic chain.

The intrinsic interest of these phenomena together with their practical importance in relation to human exposure to vibration led us to try to verify and extend these findings.

Methods and Materials. Successful experiments were carried out on 5 cats, 2 of which were first decerebrated. The animals were anesthetized with ether; the quadriceps femoris was exposed together with its tendon, nerve and vascular supply using great care not to damage these structures. Mechanical vibration was applied using a permanent magnet loud speaker from which the cone had been removed and to which a rod 6" long x  $\frac{1}{4}$ " diameter had been fixed. The device was driven by an audio oscillator and a 15 watt public address system amplifier. It was mounted on an adjustable stand over the animal operating board so that it could be lowered vertically into contact with the part to be stimulated. Observations of the wave form of the vibrator indicated roughly 10 to 20% harmonic content. Frequencies of 40 to 600 cps were used effectively. The maximum amplitude attainable at frequencies below 100 cps was about  $1\frac{1}{2}$  millimeters with less at the high frequencies. Most of the results were obtained at 100 cps., 1 millimeter amplitude. The patellar reflex was elicited by steady, controlled



tapping of the tendon. Observation was visual and no recordings have yet been made.

The decerebrate animals were further prepared to permit obtaining contraction of the quadriceps by elicitation of the crossed extensor reflex.

Results. The results obtained agree only in part with those of Loeckle. When the vibrator was applied directly to the belly of the muscle or to the tendon of the femur, the knee jerk was much reduced or was abolished entirely. The muscle was seen to undergo a slight contraction which persisted as long as the vibrator was applied. In the decerebrate preparations the crossed extensor response was unaffected by the vibration so that a pronounced contraction of the quadriceps could be stimulating the opposite sciatic nerve at a time when tapping the tendon was ineffective. Application of the vibrator to the freed artery had no observable effect on the reflex. In one case the vibrator was applied to the artery in situ with a resulting diminution of the reflex. These responses appeared to be readily reproducible.

Discussion. A few additional points seem relevant. Echlin and Fessard (3) recorded nerve action potential during application of a vibrating tuning fork (100 to 440 cps) to a muscle, its tendon, and its bony support. These potentials were synchronized with the vibration. Sommer (4) obtained electromyograms from the biceps of the vibrated arm of a man (37cps). These potentials were also synchronized with the vibrations. On the other hand, Loeckle's electromyograms showed no such impulses.

One is thus faced with sets of observations which are difficult to reconcile. If Loeckle's findings are discounted, an explanation of the inhibitory effect of vibration can be given without directly invoking autonomic pathways. The vibration produces a periodic synchronous stretch reflex in which most of the muscle receptors are involved. There is thus a slight tetanic contraction of the muscle, and tapping the tendon is now ineffective since the reflex arc is in steady use. However, this does not involve all the motor neurons, and muscular contraction is obtainable via other routes, in this case, the crossed extensors pathways.

If on the other hand, it be assumed that the results of Echlin and Fessard, and of Sommer, are artifacts and that the experiments reported here constitute failures of technique, it appears to follow that vibratory stimulation of autonomic fibers may have a marked inhibitory effect on the stretch reflex. This is rather surprising in view of the generally accepted role of the sympathetic nerves as enhancing muscular activity.

It is evident that both alternatives are oversimplified, and equally evident that further careful experimentation will be necessary in order to gain an understanding of the relationships of the autonomic nervous system to the phenomena described here. Such experimentation is underway.

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## CONCEPTS CONCERNING EFFECTS ON MAN OF ABRUPT

### DECELERATIONS

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A 160 lb. man traveling at 100 mph has a momentum of 750 lb/secs. In order to bring him to rest an impulse of 750 lb/secs. must be applied to him. Under many conditions the application of this impulse is perfectly harmless, under others it is fatal. The question arises as to whether the conditions which prevail in an aircraft crash necessitate a fatal result. This depends upon the course of the distribution of the impulse between its factors, time and force, and upon the area of application of the force.

In those cases in which man has not been restrained but allowed to smash into structure the outcome has been fatal. In those cases where the man was well restrained and protected from collision with structure the rare fatalities can be ascribed to correctible features of the restraint. Since restraint has proved so valuable in providing protection from injury it is natural to question just how far this protection can be expected to operate.

After the limit of protection which can be provided by restraint and support has been reached still further protection can be afforded by utilizing energy absorbing devices. Whether or not this is necessary depends upon the tolerance of man to deceleration under aircraft crash conditions.

In order to investigate his limit of tolerance it is necessary to expose experimental animals and finally man to these decelerations, to observe the injuries which occur and to develop methods of restraint and support which afford the maximal protection from these injuries.

The injuries which are to be expected may be studied by separating external tissue trauma from internal tissue trauma and physiological dysfunction. This can be done by avoiding all external tissue trauma during the deceleration and demonstrating the internal damage and physiological effects. To do this it is proposed that test animals be decelerated while submerged in water.

Since the effects will be different depending upon the course of deceleration over time it is necessary that the experimental conditions closely approximate those of an actual crash. First, however, the deceleration time pattern which occurs in a crash must be investigated.

In the design of the experimental approach to this problem which has been developed at Naval Medical Research Institute, it is proposed to use the same device for investigating the type of deceleration time pattern occurring and for reproducing this pattern under conditions favorable for investigating the effects on experimental animals and on man. This device is essentially a 500 ft. vertical tower where velocity is smoothly gained as the test object falls freely. Deceleration is accomplished by a hydraulic method utilizing principles well known in terminal ballistics.

Although this experimental approach is required for a valid quantitative determination of the limit of human tolerance to decelerations such as occur in aircraft crashes it is encouraging to examine certain facts in an effort to determine the region in which this limit should be found to exist.

There is good evidence at the present time that a man can be expected to survive a severe crash at 100 mph in most instances with no protection other than adequate support and restraint. With energy absorbing devices much greater crash speeds should be survivable.

This evidence is gained by a consideration of the forces involved and of man's ability to survive these forces as demonstrated by such things as freak survivals in falls from great heights. Additional data on the effects of high, localized, abruptly applied pressures is available from a study of the snubbing action of safety belts in private plane crashes. In these crashes safety belts are frequently broken by the impact of a man's hips, and yet no local trauma is found. To evaluate the forces operating in these cases we have conducted a series of tests in which the strain was measured in safety belts when broken under conditions closely simulating a crash. The pressures calculated are in the range expected to occur in crashes of military aircraft.



## THE ROLE OF OXALATES IN RAT DENTAL CARIES\*

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In 1943, McClure (1) first experimentally demonstrated an in vivo destructive action of rat molar enamel by acid beverages and certain dilute acids. Investigations conducted by McCay and his collaborators supported McClure's observations in the rat and hamster (2). Such enamel etching also occurred to some extent in the teeth of monkeys and dogs (3).

It was while testing the effect of oxalic acid beverages of pH 2.6 in vivo that Gortner, et al., noticed no enamel destruction of rats' molar teeth. Instead of enamel etching, a thin film of some substance was deposited upon the crowns of the molars. Further investigations by these same workers revealed that when oxalic acid was added to other acid beverages known to cause dissolution of rats' enamel in vivo, no etching of enamel occurred. Similar results were obtained with dehydrated rhubarb and spinach which are known to contain oxalates in combined and uncombined form (4). Oxalic acid seemed to protect the teeth against such destructive effect by virtue of the amount of deposit upon the teeth. When the deposit was scraped off, the underlying enamel appeared normal on gross examination. Moreover, in vitro studies conducted by Buonocore and Bibby in 1945, on the effect of various ions on enamel solubility, demonstrated little if any enamel dissolution by oxalic acid (5).

It has been a general clinical observation of naval dental officers attached to various stations in the South Pacific, that little dental caries but large amounts of dental calculus is characteristic in natives subsisting on native foods. This is particularly true of natives in American Samoa and the Territory of Hawaii. Among the basic native foods of these people is poi (a native preparation of taro root). Jones, Larsen and Pritchard have shown that in the older natives who eat foods

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\*The material in this article should be construed only as the personal opinions of the writers and not as representing the opinion of the Navy Department officially.



containing a substantial amount of poi, little if any dental caries occurred (6). It is interesting to note that taro flour (known to contain oxalates) also produced characteristic deposits on the molars of white rats (7).

Since several investigators have observed that dental caries is seldom found under calculus in human teeth (8), and since oxalates seem to play a role in calculus deposition, an investigation was undertaken to determine the effect of these substances on the carious process.

### Procedure

One hundred sixty-five weanling white rat litter mates were divided into five major groups, hereinafter identified as groups 1 to 5, inclusive. Each of these groups was further divided into three subgroups, hereinafter designated subgroups A, B, and C. Litter mates of random parents were used for groups 1, 2, and 3. Groups 4 and 5 were litter mate offspring from four pairs of brother and sister matings from the same litter.

Subgroup A in each of the major groups acted as controls and received the coarse corn caries-producing diet recommended by McClure (9). Subgroup B in the major groups received the caries diet containing a small quantity of added oxalates in different forms. Subgroup C in the major groups received the same caries diet but with the quantity of added oxalate 5 to 10 times that of subgroup B. Since the oxalic acid equivalent necessary to produce protection against acid beverage has been shown to be 0.11% or more (4), this quantity was accepted as a reference value from which the effect of increases or decreases in the dietary oxalates could be determined.

The oxalates were administered as follows: In group 1, subgroups B and C, the caries-producing diet contained 0.02% and 0.11% sodium oxalate. Group 2, subgroups B and C, were fed a caries-producing diet containing 0.06% and 0.33% sodium oxalate. Rats in group 3, subgroups B and C, received the oxalates in their caries diet in the form of dehydrated spinach 1.6% and 8%, respectively (dehydrated spinach contains about 5.88% oxalates in combined and uncombined form). Group 4, subgroups B and C, received Purina lab chow and 0.2% and 0.22% potassium oxalate in distilled water to drink ad libitum (which the rats did adequately), 10 days prior to being placed on a caries-producing diet, the oxalate containing water being continued for the entire experimental period. In group 5, subgroups B and C, the same regimen was followed as in group 4 except that the concentration of potassium oxalate in the drinking water was 0.0002 and 0.002%, respectively, which was consumed on the average of 60 cc. daily. The white rats remained on the caries-producing diet for 100 days, whereupon the animals were sacrificed. The heads were removed and autoclaved, after which all tissue, except the jaws with their teeth, was discarded. The jaws were placed in ether and then 95% ethanol, about five minutes in each. This was repeated after which the jaws were dried by evaporation (10).

The teeth of each half jaw were ground in an occluso-cervical direction. A procedure of alternate grinding and inspection (recording pertinent data) was followed (10) until approximately 15 to 20 planes had been examined. In this way the caries incidence and extent could be determined through the various planes. The scoring system used to record extent of the carious lesion is a modification (10) of that adopted by Cox, et al. (11) for occlusal caries in the white rat.

### Results

In groups 1 and 2, the incidence of dental caries in 57 white rats, 100 days on a coarse corn caries-producing diet to which varying amounts of sodium oxalate had been added, ranged between a mean of 14.1 and 19.5 carious lesions. The mean total extent of these lesions varied between a score of 33.5 and 46.9. The effect of adding the oxalates in the form of dehydrated spinach to the caries diet of 30 white rats in group 3 was: mean caries incidence between 17.3 and 19.5 lesions of mean total extent between a score of 33.5 and 33.1. Thirty rats in groups 4 and 5 on a caries diet who received the oxalates in their drinking water in varying amounts, showed a mean number of carious lesions ranging between 17.9 and 21.6. Their mean total extent varied between a score of 34.2 and 43.3. The figures given include the caries occurrence in the controls.

After applying a technique for analysis of variance to the results obtained in the different groups of experimental animals, it became evident that the oxalates as given exerted no influence on the dental caries experience. In group 3 (dehydrated spinach) the significant difference in the caries incidence and extent between rats on the different diets was accountable on a hereditary rather than dietary basis upon statistical analysis. In none of the other major groups of experimental animals could an hereditary influence on the occurrence of dental caries be demonstrated statistically. Neither the concentration of the oxalates in the diet (0.02% to 0.33%) nor in the drinking water (1:1,000 to 1:1,000,000 oxalic acid equivalent) seemed to have any effect on caries in the groups of white rats studied.

No significant difference could be found between the caries occurrence in those 5 subgroups A of white rats which were used as controls, all of which subgroups were on the same caries diet. The mean carious lesions ranged between 14.1 and 20.6 and their total extent varied between a mean score of 35.1 and 42.0.

Many carious lesions were found immediately under the dental calculus-like deposits within the coronal grooves. That these lesions may have been extensions from other planes reaching areas immediately under the deposits could be dismissed since the system of alternate grinding and inspection through all planes precluded such a possibility.



## Discussion

In this study it is evident that the oxalates in the dietary are not protective against the dental caries process as induced by a caries diet in white rats. Similar incomplete unpublished data, thus far accumulated on cotton rats here studied, tend to show the same results, although it is not implied that similar results are to be expected in humans.

The clinical differences between acid etching or erosin, and dental caries have been generally observed by practicing dentists and investigators working with experimental animals. Grossly reviewed, these are the loss of tooth substance leaving a hard polished exposed surface discolored to varying degrees and depth in erosin or acid etching, as compared to the chalky appearance and hardness of initial carious lesions progressing to the leathery, discolored, extensive destruction of tooth substance in advanced dental caries. Since the oxalates have been shown to be protective against etching of rat molars by acid beverages (4), and not against the carious process, it appears that this study is supportive evidence in the differentiation between acid erosin and dental caries.

The fact that the "oxalate" deposits in the coronal grooves of the experimental rats did not influence the carious process, and that caries was seldom found under human dental calculus (8), raises a question of chemical composition of both types of deposits. Such comparisons have as yet not been made. It is possible that the chemical composition of the "oxalate" deposits may not be the same as other dental calculi; and that, consequently, some factor or factors inhibiting dental caries under human dental calculus may be absent under the "oxalate" deposits. Unless, of course, we may be ignoring a physico-chemical mechanism which might be operating peculiar to rat molars and associated with their external configuration.

Concerning the mechanism involved in the process of initial dental caries, there appear to be two schools of thought. The one headed by Miller (15) who contend that decalcification of enamel caused by products of oral acidogenic organisms, occurs first and is followed by proteolysis of the inorganic content of enamel or dentin by oral proteolytic bacteria. The other school headed by Hopewell-Smith (16) considered the sequence of events to be proteolysis of the enamel cuticle (Nasmyth's membrane) by oral proteolytic organisms followed by the decalcification process in the enamel by acidogenic bacteria. The results of the study on rats here undertaken would tend to minimize the acidogenic factor as most important in the initial stages of the dental caries process. Were the acid dissolution of the enamel of greatest importance in the initial stages of dental caries in general, then the protective action of the oxalates should have reduced caries occurrence and extent in a manner similar to the way they protected the enamel against the solvent effect of acid beverages shown in other studies (4). As has been shown, the dietary oxalates did not influence dental caries in rat molars.



From this work it becomes obvious that further work will be necessary to more fully evaluate the factors tending to operate in initial dental caries.

### Summary

1. The oxalates administered in the diet and drinking water in this study produced no significant effect on the dental caries incidence and extent in five groups of white rat litter mates fed a coarse corn caries-producing diet.
2. Although considerable dental calculus-like deposits occurred within the grooves of the experimental rats' teeth, they did not appear to influence dental caries occurrence in these susceptible areas.
3. This work tends to minimize the importance of the acidogenic factor in the initial stages of the dental caries process.
4. Further in vivo studies in this problem seem indicated to determine if any variations exist in different species and different time intervals of application of oxalates prior to placing animals on a caries producing diet.

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## REDUCTION OF CENTRAL HYPER-IRRITABILITY FOLLOWING BLOCK ANESTHESIA OF PERIPHERAL NERVE

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The purpose of this study was to determine whether hyperirritability of the dental innervation, produced by previous painful stimulation of the teeth, could be permanently reduced to a normal level of irritability by a single application of procaine block.

This question arose from two previous studies (1), (2). In the first (1) it was observed that the most prevalent cause of dental pain engendered by alteration of barometric pressure (aerodontalgia) was referred pain from an occluded paranasal (probably maxillary) sinus. Not only was this pain referred to the teeth, but in an overwhelming majority of cases, it was referred to teeth which showed evidence of previous painful stimulation as by filling, trauma, caries or extraction.

This led to the hypothesis that the mechanism involved in the referral of pain in this case was one akin to spatial summation of afferent stimuli in the motor reflex and that the stimulus arising from the sinus only "facilitated" a chronic sub-threshold stimulus from the offending tooth. Since the injury to the teeth in this study was in many cases made years previous to the observation the authors were intrigued by this evidence of a prolonged "central excitatory state". Thus a second study (2) was set-up to determine whether this observation could be repeated under conditions whereby all known factors could be controlled. Fillings and extractions were made without block anesthesia (NO was used for extractions) and after an interval of several weeks the nasal epithelium was stimulated mechanically, by a needle prick, in the region of the maxillary sinus ostium. With a single exception, pain was referred to the previously stimulated teeth whether or not any sensation was evoked from the site of the immediate stimulation.

Collateral observations made in the above studies indicated that hyperesthesia does not exist in teeth receiving dental treatment under the influence of procaine block anesthesia. Thus this study was set-up to determine whether procaine block is efficacious in removing an already established hyperesthesia.



## Subjects and Methods

The subjects reported on in this paper were the same as those used in a previous investigation (2). These had undergone dental treatment of approximately bilateral symmetry either without anesthesia, or under general nitrous oxide anesthesia. Following this treatment all subjects exhibited a tendency for occurrence of pain in the treated teeth elicited by mechanical stimulation of the ipsilateral maxillary ostium.

Since the subjects had received approximately equivalent treatment bilaterally, it was decided that the upper and lower quadrants of the dextral dentition could be used to test the efficacy of procaine anesthesia in eliminating this susceptibility to referral of pain, while the sinistral side could be left unanesthetized as a control.

In order to determine whether elevated irritability was still existent, the right maxillary ostium was mechanically stimulated, by means of a needle prick, and the occurrence of pain as reported by the subject recorded. Following this procedure, block anesthesia of the dentition in the area to which pain was referred was produced, using an injection of 2% solution of procaine hydrochloride with epinephrine in a concentration of 1 part in 50,000.

The anatomical locations of the blocks were as follows;

1. For the maxillary dentition, infraorbital (anterior and middle superior alveolar branches of the maxillary division of the trigeminal nerve); infratemporal (posterior superior alveolar branches of the maxillary division of the trigeminal nerve), greater palatine and nasopalatine blocks were used.

2. For the mandibular teeth, the mandibular or inferior alveolar block was used (lingual nerve before it enters the region of the floor of the mouth and the inferior alveolar before it enters the mandibular canal at the lingula). This was supplemented by block of the buccinator nerve.

Approximately 2 weeks (10 to 16 days) after production of the temporary block both maxillary ostia were stimulated and the incidence of tooth pain recorded.

The results of stimulation of the maxillary sinus ostia are given in table 1.

From table 1 it may be seen that mechanical stimulation of the maxillary sinus ostium was effective in eliciting tooth pain in dextral teeth in 100% of cases before anesthesia.

Approximately 2 weeks (10 to 16 days) after use of procaine block anesthesia, no referred pain to dextral teeth could be produced by ipsilateral maxillary ostium stimulation. The "control" sinistral teeth still responded with strong pain sensation upon stimulation of the ipsilateral maxillary ostium in 13 of 14 subjects (93% of cases).

Table 1 - Experimental Data

Subject	Dental	Work	Interval	Dental	Anes-	Interval	Dental	Interval
		Proc <sup>1</sup>	1	Pain	thesia	2	Pain	3
Sex Age	Tooth		Days	Stim-1	Used	Days	Stim-2	Days
M 23	3	11	49	+	+	13	-	62
	14	1			-		+	62
M 14	4	1	53	+	+	12	-	65
	14	1			-		+	65
M 52	3	1	55	+	+	11	-	66
	14	11			-		+	66
M 28	2	1	52	+	+	11	-	63
	13	11			-		+	63
M 21	3	11	30	+	+	11	-	41
	14	1			-		+	41
M 24	4	11	50	+	+	13	-	63
	14	11			-		+	63
M 22	3	11	46	+	+	11	-	56
	13, 14	11, 1			-		+	56
M 22	3	11	59	+	+	10	-	69
	13	1			-		+	69
F 28	2, 3	11, 1	53	+	+	15	-	68
	15	1			-		+	68
F 23	3	11	50	+	+	16	-	66
	14	1			-		+	66
F 22	4	V	53	+	+	16	-	70
	13	11			-		+	70
M 30	3	11	53	+	+	14	-	67
	13, 14	1, 1			-		+	67
M 27	20	11	38	+	+	16	-	54
	30, 31	1, 1			-		+	54
F 28	3	Ext.	37	+	+	11	-	48
	13, 14, 15	Ext.			-		+	48

<sup>1</sup>Fillings according to Black's (1936) classification. 1-Occlusal surface. 11-Mesio- or disto-occlusal surfaces. V-Gingival surface. Ext-Extraction.

Interval 1. Days elapsing between original dental treatment and stimulation of maxillary sinus ostium just prior to procaine injection.

Interval 2. Days elapsing between procaine injection and final stimulation of maxillary sinus ostium.

Interval 3. Total time (days) elapsing between original dental treatment and final maxillary stimulation.



## Discussion

From a standpoint of practical dentistry, the observations reported present a simple method of alleviating tooth pain referred from the maxillary sinus.

Of equal importance from a fundamental standpoint are the implications of these results with respect to the neurophysiology of pain mechanisms. The authors are very fortunate in this instance in having available an example of "pain facilitation" in which both sources of stimulation are known and capable of manipulation. Denslow and his co-workers (3), (4), have reported a closely allied condition in the case of pain perception from pressure on the spinous processes of the thoracic vertebrae. In their work a considerable advantage is reported from the presence of motor activity associated with pain transmission. This allows collection of objective evidence of neural activity which is much more satisfactory in many respects than the purely subjective responses upon which the present paper is based. Nevertheless, the authors believe that subjective evidence of positive character such as the dental pain used as the criterion for this study allows a high degree of confidence in the results.

Previous work by Hardy et al. (5), Denslow, (3), (4), Livingston (6), Heinbecker (7) and the present authors (1) and (2) has indicated that referred pain may be due to facilitation of a sensory pathway maintained at an elevated level of irritability, by stimulation of another sensory pathway in the same segment. Although this observation is fairly well established, (5) there has been relatively little insight into the mechanism by which the elevated central irritability is maintained. Some workers, Sherrington (8), and Livingston (6), have suggested a "vicious circle" concept involving afferent autonomic pathways which maintain the tissue in the region of the pain receptor in a disturbed physiologic state. Some evidence for this is found in the cases of "phantom limb pain", causalgia and other similar conditions. Denslow reports a "doughy consistency" of the tissue in the area of vertebral spinous processes with low pain thresholds.

In the present work, two possibilities for maintenance of elevated irritability are manifest. The simplest case would be that of a disturbed physiologic condition of the dental pulp due directly to the presence of filling materials, anatomical changes, etc., in the immediate vicinity. Another possibility is that of a disturbed circulation to the dental pulp resulting from autonomic activity in concurrence with the "vicious circle" concept. The observations reported in the present paper indicate that the latter is the more likely explanation since reduction of the elevated irritability is effective for as long as 16 days when accomplished by a single anesthetic block of the innervation. If the elevated irritability were due to reactions continuously produced by irritating agents or structures in the dentition, one would expect that symptomology would return immediately on recovery from anesthesia.



Further, it appears from the foregoing observations that the receptor neuron itself is an important factor in maintaining an elevated central irritability. This does not discount, however, the possible importance of the autonomic innervation in maintaining a localized tissue disturbance at the site of the receptor endings.

### Conclusions

1. An elevated central irritability is maintained in the sensory pathway in teeth undergoing dental treatment without local anesthesia for a long period (two months in the present paper, but previous work indicates that this may exist for several years).
2. The elevated central irritability may be reduced for an extended period by temporary procaine block anesthesia of the innervation.
3. Since referred pain to the teeth from the maxillary sinus depends upon elevated irritability of the dental sensory pathway, this procedure eliminates further dental pain of this origin either permanently or for a considerable period of time.
4. The afferent peripheral neuron itself is an important unit in the maintenance of elevated central irritability.

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BIOLOGICAL MEASUREMENTS AS A BASIS FOR ESTABLISHING  
THE DIMENSIONS OF THE COCKPIT WORKING AREA FOR THE  
OPERATION OF MANUAL CONTROLS IN AIRCRAFT

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One of the most fundamental biological problems in cockpit design is the location of aircraft controls to permit the pilot maximum effectiveness in the operation of his aircraft. Controls should be within easy reach without requiring major movement of the pilot's body; further, they should be placed so that the muscular movement required for their operation can be carried out efficiently.

In current aircraft, the pilot is obliged to make major movements which may result in losing sight of his instruments momentarily, altering the pressure on flight controls and increasing the difficulty in interpreting his orientation in space. Each of these factors increases the pilot's chances of getting into trouble. Further, current design markedly reduces the possibility of obtaining a reasonable benefit from shoulder harness in the event of a crash, since the major movements essential to reach the controls cannot be carried out when the shoulder harness is locked, and are impeded even when the shoulder harness is worn in the unlocked position.

Experimental Procedure. A study was undertaken at the Naval Medical Research Institute to determine how far a man in a fixed position can reach in various directions into the surrounding area. Arm reach measurements were made on 139 subjects. Seventy-nine were naval aviators, 45 from transport squadrons and 34 from fighter squadrons. The remaining 60 were non-pilot personnel, 10 officers and 50 enlisted men who served either as members of the staff of the Naval Medical

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Research Institute or as volunteer subjects. Anthropometric measurements were taken to provide a description of the men used in these studies and to serve as a basis of comparison of this sample with other series describing the body size of men in the armed services.

The testing apparatus for the reach measurements consisted of a Warren-MacArthur seat mounted on a platform and equipped with suitable horizontal and vertical measuring rods. The horizontal arm is pivoted at a point directly above the reference point of the seat. This reference point is taken as the upper level of the seat cushion at its line of intersection with the small lower cushions of the back pad. It corresponds to, or very closely approximates, the reference point used by the Army.

Shoulder straps and the safety belt were secured during the reach measurement tests. The subject was instructed to keep his back against the seat cushion in an easy sitting posture. He reached for and touched successive points on the vertical measuring rod as it was brought toward him by moving it along its horizontal support. Horizontal distances from a vertical line rising from the seat reference point to the vertical measuring rod at extreme finger tip reach were measured for each of 99 points. Measurements were made at 6-inch vertical intervals from 52 inches above to 8 inches below seat level at  $0^{\circ}$  and at  $15^{\circ}$ ,  $45^{\circ}$ ,  $75^{\circ}$  and  $105^{\circ}$  to the right and left of the mid line.

Results. The over-all pattern is such that the average reach increases as the arm is moved from zero to  $15^{\circ}$ ,  $45^{\circ}$ ,  $75^{\circ}$  and  $105^{\circ}$  to the right, and decreases as the arm is raised or lowered from the trunk height of the subject. The unequal radii of any arc describing various successive points of reach are attributable to the position of the shoulder joint in relation to the midsagittal plane of the body, and to its architecture.

If the arms are extended forward, they intercept an arc at approximately  $12^{\circ}$  to  $15^{\circ}$  to the right and left of the mid line. As the arm is moved more and more laterally, one-half of the chest breadth (at shoulder height) contributes increasing increments to the reach dimension until the arms are extended straight out from the sides. Further, the tissues lying behind the shoulder contribute to arm reach at points forward of this joint; the extent of this contribution decreases continuously as the arm is moved from  $15^{\circ}$  to  $90^{\circ}$  from the midsagittal plane of the body.

The distribution of arm reach measurements deviates considerably from normal so that theoretical predictions should not be made by methods used for normal distributions. Statistical analysis and study of the nature of the distribution curves, however, reveal that prediction of each reach dimension which can be obtained by 97% of the sample population of 139 subjects is justifiable. The standard deviations for the normal curves cannot be used in predicting maximum reaches for smaller percentages of the population because of the positive skewness of the curves. Data are given for the maximum distance for each position on the outer boundary of the working area for operation of manual

controls which can be reached by 97% of the population. The area has been described at various levels for angles up to 75°. The design of Navy carrier aircraft cockpits will not require extended arm reach beyond this angle. The dimensions for the areas 75° - 105°, which may be of value for defining the manual working areas in transport aircraft cockpits, may be obtained by extension of the methods used in these studies.

#### TABLE OF REACH MEASUREMENTS

The maximum distance (inches) at various points in the boundary area for operation of manual controls which can be reached by 97.73% of the population at each position and 92.9% of the NMRI series at every position; N = 139.

ANGLE (degrees)	0	R15	R45	R75
Level (inches)				
above seat				
reference point				
46	11.6	13.7	15.0	17.0
40	18.9	20.5	22.4	24.1
34	22.9	24.9	26.6	28.0
28	25.5	27.1	29.1	30.1
22	26.7	28.2	30.3	31.4
16	26.6	28.0	29.7	31.6
10	25.3	27.0	29.3	30.4
4	22.6	24.2	26.4	27.9
-2	17.5	19.7	21.8	22.8

Distances for right arm reach are measured from the vertical line through the reference point with the subjects' shoulders touching the back cushion; seat back 13° from the vertical. R15° stands for 15° to right. Reach for left arm can be outlined by using above measurements at corresponding points to the left of 0°.

The mean minus 2 standard deviations provides a finger tip reach measurement satisfactory for 97% of the NMRI series in any specified position. Since, however, the  $X - 2\sigma$  value is calculated for each position, it does not necessarily follow that identical groups of men could reach every point of the periphery of a work area of such dimensions. In order to determine the percentage which could reach every point, the reach for every individual in the series was compared with the value for  $X - 2\sigma$  at every point. These comparisons showed that 93% of the NMRI Series were able to reach the predicted value at every position.

Because of the nature of the distributions of arm reach measurements and because of numerically small but statistically significant



differences in the age, weight and bodily dimensions of random sample populations of men in the military services, it may be expected that the percentage of other sample populations that can reach specific points on the maximum boundaries of the working area will vary for different series. The absolute differences in these percentages cannot be predicted. Reaches which can be obtained by 93% of the NMRI sample may perhaps be obtained by 75% or perhaps by 98% of the men in another series. Unfortunately, we cannot get any information from the several large series of anthropometric measurements which have been reported for men in the armed services since no significant correlation has been found between a single anthropometric measurement and arm reach. Such a relation would be complex because of the relatively wide range of individual variation in arm length, sitting height, chest depth and the "rowing" of the mechanical center of movement of the shoulder. If it becomes necessary to establish more precisely percentages of sample populations which can reach any given set of dimensions, the problem can be solved more readily by direct methods.

The solution of the problem lies in the use of a device consisting of "reach shelves". This is a series of shelves, arranged at specified heights, which are cut out in accordance with the dimensions obtained as a result of these studies. Their edges describe the maximum boundaries of the working area. Such a device was constructed at this Institute and subsequently another one at the Special Devices Center, Office of Naval Research, to illustrate the results of preliminary studies on twenty subjects. The dimensions of the original models will have to be revised in the light of the final results. A modified device which permits adjustment of individual points would increase its over-all usefulness.

Tests could be conducted on a large number of pilots at training centers in order to observe the differences in the percentages of other series for which these dimensions are satisfactory. The tests would be simple, and analysis of the results would be reduced to a minimum. Each pilot would take his place in a pilot seat, located at the correct distance, and attempt to reach each of the designated points. Since scoring of the tests is based only on success or failure, the results could be presented with a minimum of delay.

Such a procedure also is readily applicable to selection of aviators; it would not materially lengthen the time normally devoted to physical examinations.

Further, changes in the seat back angle shift the boundaries of the working area up or down; other changes in seating arrangements also may influence the configuration of these boundaries. The modified "reach shelves" may be used for validation of the necessary corrections.



## METABOLIC EFFECTS OF FOLIC ACID

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The hemological effects of folic acid have been thoroughly investigated and the results have been reported in a series of papers by Doan(1), Spies(2,3), and Moore(4), with their co-workers. Practically nothing, however, has been done on the metabolic effects of this substance and the work being reported here was undertaken for the purpose of filling in this gap.

Method. Observations have been made on both normal human beings and on hospital patients suffering from disorders amenable to folic acid. Both normal subjects and hospital patients lived in the hospital and were maintained on a weighed diet. A 2- or 3-week control period was followed by a period of equal length during which 30 mg. of folic acid was given intramuscularly daily. A 4-day balance period was carried out each week. At the beginning of each balance period stool markers were given and collection of urine was begun. At the end of the 4-day period a second stool marker was given and the collection of urine discontinued. Total nitrogen was determined on the urine and on all stools between the two markers and from these data the total nitrogen excretion was calculated. An aliquot portion of diet was saved each day and analyzed for nitrogen, thus making possible the calculation of the nitrogen intake. From these two figures the nitrogen balance was calculated. In addition to the nitrogen balance, the oxygen consumption, carbon dioxide production and non protein respiratory quotient were determined, using the Tissot-Haldane technique, under the following conditions: basal (twice weekly); one hour after a standard meal and before, during and after walking on a treadmill at the rate of approximately 1.3 miles per hour.

Results. Results obtained on 7 normal controlled subjects are shown in table 1. In column 1 is shown the daily caloric intake before and after the administration of folic acid. Each of these figures is based on the average of two or three balance periods. It is to be noted that in every case the caloric intake was slightly greater after folic acid than before. These differences are slight and result from the fact that readjustments of the caloric intake were made in an effort to prevent the loss of weight by the subjects and to keep the appetite satisfied. The basal oxygen consumption in cc. per minute is shown in column 2. In no case was there a significant variation in this value after the administration of folic acid. The same statement holds true for basal calories per hour which are listed in column 3, since these figures were calculated directly from the oxygen consumption. In columns 4, 5 and 6, the percentages of total basal



calories derived from proteins, carbohydrates and fats are listed. Five of the 7 subjects showed only a negligible change in the per cent of total calories derived from protein following the administration of folic acid. One subject showed an increase of about 6% while the remaining one showed a drop of 5% in this figure. We must conclude, therefore, that folic acid has no significant effect upon the utilization of protein as a source of calories. On the other hand, 6 of the 7 subjects showed a marked increase in the percentage of total calories derived from carbohydrate after the administration of folic acid. These 6 subjects showed an average increase of more than 10% in the percentage of total calories derived from carbohydrate. There was, of course, a corresponding decrease in the percentage of calories derived from fat. This seems to indicate that folic acid has a tendency to increase utilization of carbohydrate. In column 7 the average excretion of nitrogen per 24 hours is given in grams while in column 8 the intake of nitrogen is similarly listed. In column 9 are listed the nitrogen balances in grams per 24 hours. These nitrogen balances on the whole seem rather large and there may be a systematic error involved in their calculation. However, the administration of folic acid produced no significant difference in the figure and we must, therefore, conclude that this substance does not affect the retention of nitrogen in normal subjects. In column 10, the specific dynamic action is tabulated in terms of percentage increase in total calories  $1\frac{1}{2}$  hours after the ingestion of a standard meal. It will be noted that 3 of these subjects, L. P., D. Y., and R. H., showed a definite increase in their specific dynamic action during the administration of folic acid. On the other hand the specific dynamic action with subject, S. J. dropped from a figure of 43% preceding the administration of folic acid to 37% afterwards. The other three subjects showed only small variations, 3% or less, in this figure. It would appear therefore that folic acid does not have any consistent effect on the specific dynamic action in normal subjects. The results obtained on three hospital patients are shown in table 2.

In column 1 of this table is shown the caloric intake of each of the 3 patients before and after the administration of folic acid. It will be noted that following the administration of folic acid this figure increased significantly with all 3 subjects, the increase varying from 15.1% in subject V. M. to 31.6% in H. D., the average increase being 21.2%. This is much greater than the corresponding increase in caloric intake noted in the control subjects. Among the controls the increase varied from 0.9% in subject A. E. to 10.2% in subject J. V., the average for the 7 controls being 6.1%, less than 1/3 of the figure for the hospital patients. These figures justify the conclusions that folic acid stimulates the appetite and tends to increase the total caloric intake. The basal oxygen consumption and basal calories per hour as shown in columns 2 and 3 decreased slightly, following the administration of folic acid. Thus, of 7 controls and 3 hospital patients only two failed to show a decrease in basal oxygen consumption, but this decrease is so slight as to be of questionable significance. The effect on utilization of carbohydrate is less pronounced among the hospital patients than it was among the normal controls. Two of the 3 patients showed increases of  $6\frac{1}{2}$  and 7% respectively, while the third subject, A. M., showed a decrease of nearly 8% in the utilization of carbohydrates. As was the case with the normal controls, all hospital patients

showed an increase in retention of nitrogen following the administration of folic acid. The specific dynamic action of a standard meal also increased with all 3 hospital patients. Since the specific dynamic action was increased only in about half of the control subjects the effect on this figure among control subjects would seem to be in doubt.

In summary, we may say that these data seem to indicate that folic acid has the following effects: it improves the appetite with a resultant increase in total calories consumed and increases retention of nitrogen; an effect tending to increase the utilization of carbohydrates seems probable; while there is a tendency for the specific dynamic action of food to be increased with hospital patients, there is also the possibility of a slight effect in decreasing the basal oxygen consumption.

The work which is being reported is a cooperative enterprise involving personnel from the Departments of Medicine, Physiology, and Hospital Dietetics, and is being done under contract with the Office of Naval Research. Personnel taking part in the research are Dr. Benjamin C. Houghton, of the Department of Medicine, Mrs. Martha Lewis and Miss Julia Trossbach of the Department of Hospital Dietetics, Drs. R. C. Grubbs, Clifford Angerer and F. A. Hitchcock, of the Department of Physiology.

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Table 1. Metabolic Data on Normal Controls

Subject		1	3	4	5	6	7	8	9	10
Daily intake (cal.)	Basal cal. per hr.	% total calories (basal) from			N		N		S.D.A. (%)	
		Prot.	CHO	Fat	exor. per 24 hr. (gm.)	intake per 24 hr. (gm.)	bal. per 24 hr. (gm.)			
S. J.	B	3308	59.54	16.9	38.5	44.6	9.2	21.2	12.0	43
	A	3406	60.81	22.7	56.0	21.3	11.9	20.7	8.8	37
J. V.	B	2320	61.80	19.8	31.0	49.2	12.7	17.9	5.2	31
	A	2557	60.98	19.6	39.7	40.7	13.8	18.5	4.7	32
L. P.	B	2714	61.64	22.6	45.2	32.2	14.9	18.7	3.8	24
	A	2846	58.86	21.4	53.4	25.2	15.2	17.8	2.6	38
D. Y.	B	2714	62.44	21.8	27.6	50.6	16.1	18.7	2.6	37
	A	2846	60.56	22.8	39.6	37.6	15.6	17.8	2.2	42
A. E.	B	2173	73.35	22.9	20.8	56.3	15.7	17.2	1.5	27
	A	2193	73.18	17.8	35.2	47.0	15.6	16.6	1.0	25
R. H.	B	2228	56.57	32.1	30.0	37.9	14.1	15.3	1.2	27
	A	2450	56.25	32.4	34.7	32.9	14.5	15.9	1.4	36
H. F.	B	2228	59.18	24.9	39.4	35.7	12.8	15.3	2.5	26
	A	2420	58.71	23.4	38.6	38.0	13.3	15.5	2.2	23

Table 2. Metabolic Data on Hospital Patients

Subject	1	3	4	5	6	7	8	9	10								
										Daily caloric intake (cal.)	Basal cal. per hr.	% total calories (basal) from		N excr. per 24 hr. (gm.)	N intake per 24 hr. (gm.)	N bal. per 24 hr. (gm.)	S.D.A. (%)
												Prot.	CHO				
H. D.	B	1503	55.4	13.0	7.0	80.0	13.2	9.9	-3.3	7.5							
	A	1978	51.7	15.3	13.5	71.2	9.6	13.3	3.7	20.0							
A. M.	B	2342	78.4	9.7	37.5	52.8	9.8	14.4	4.6	12.5							
	A	2727	76.3	9.7	29.6	60.7	7.7	15.2	7.5	20.0							
V. M.	B	1864	65.2	10.7	32.8	56.5	12.6	12.9	0.3	17.5							
	A	2146	65.0	12.3	39.8	47.9	12.1	14.4	2.3	25.5							





THE USE OF FLUORESCEIN IN STUDIES OF THE PIAL  
CIRCULATION IN MONKEYS WITH A NEW TYPE OF  
LUCITE CALVARIUM

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The procedure for preparation of the lucite calvarium in the monkey as originally described by Pudenz and Sheldon has been modified to the extent that the lucite calvarium is fixed to a metal ring which in turn is screwed to the skull. A 4-stage operative procedure is required, in which the first two stages involve preparation and implantation of the ticonium ring, while the last two are essentially the same as described by the original authors. The advantages of the procedure are chiefly that it permits more reliable control of cerebrospinal fluid leakage, and secondly, since the calvarium is removable, the preparation is adaptable to wider experimental application.

Fluorescein, injected intravenously into prepared monkeys, passes rapidly through the pial vessels without staining the cerebral surface. This dye, being non-toxic and rapidly eliminated from the blood can be used to measure not only the circulation time to the brain, but also the time relationships of blood flow in the pial circuit.

Three photographic procedures have been devised to record the emitted light from the dye during its appearance and passage through the brain vessels. The first is by time exposure with standard equipment; the second is by instantaneous exposure with filtered light from a flash bulb; and the third is sequence photography with a 35 mm. motion picture camera driven by a constant speed motor, and loaded with high speed film.

Density measurements of the photographic negatives obtained in sequence, permit graphic representation of the time relationships of the dye in its course through the vessels.

An automatic syringe which injects intravenously a known amount of dye at constant pressure, simultaneously indicating the beginning of the injection on the photographic record, is described. The combination of this device together with photographic recording of the appearance of the dye in the cerebral vessels constitutes a completely objective method for measuring circulation time. Preliminary data obtained with this apparatus will be presented.



# CORONARY SINUS CATHETERIZATION FOR STUDYING CORONARY BLOOD FLOW AND MYOCARDIAL METABOLISM

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The Forssman intravenous catheter (1), as modified by Cournand (2,3) has permitted the study of blood flow and metabolism of several important organs, previously inaccessible in both man and fully intact animals. Catheter technique has not yet been extensively applied to the study of the coronary circulation, although catheterization of the coronary sinus of man, without apparent damage, has been recently reported by Sosman and Dexter (4) and by Bing and coworkers (5). Coronary sinus catheterization, if proven to be safe, would yield particularly valuable information concerning cardiac physiology if it were possible at the same time to measure coronary blood flow, and hence the cardiac efficiency and total cardiac exchange of various metabolites.

Harrison and coworkers (6) inserted a modified Morawitz brass cannula (7) through the external jugular vein into the coronary sinus of intact morphinized dogs. By occluding the sinus with a balloon, they diverted all of the outflow for direct measurement, and observed the effects of acute experimental heart failure on cardiac efficiency. However, the absolute volume of sinus outflow is an inconstant fraction of total coronary flow (8,9), and the Morawitz cannula is too traumatic for use in man. Other methods available for measuring coronary blood flow, as recently reviewed (10,11), require major operative procedures for installation of recording devices.

The nitrous oxide blood flow method, however, developed by Kety and Schmidt for measuring cerebral blood flow (12), has provided an added incentive to develop a technique for catheterizing the coronary sinus, as noted in preliminary reports (13,14). To measure mean coronary flow through the left ventricular myocardium, this method requires only representative samples of coronary sinus blood obtained through a non-occluding catheter, along with arterial blood samples, during nitrous oxide inhalation. Preliminary application of the method to the coronary circulation has already been reported by Eckenhoff and coworkers in open chest experiments (15).

A systematic coronary sinus catheterization technique, as reported here, was therefore developed not only to permit the study of coronary blood flow and myocardial metabolism in intact dogs, but also to determine the practicality and possible hazards of a similar procedure in man.



## Methods

Anesthesia and General Observations. Pentobarbital sodium, 25 mg./kg., was injected intravenously before the procedure, and the dog was maintained in a stage of light anesthesia, with an active lid reflex, a fairly constant blood pressure, and a respiratory rate and pulmonary ventilation adequate to insure a normal arterial oxygen saturation.

Although comparable catheterization procedures have been done in unanesthetized animals (17,18), anesthesia was used in this study chiefly for convenience, and to provide a reasonably reproducible and steady status of the circulation (18,19).

Arterial hematocrit and oxyhemoglobin content were also measured frequently during each experiment. Oxyhemoglobin values were standardized against simultaneous oxygen capacities as measured by Sendroy (20). Oxyhemoglobin and arterial oxygen contents were used to determine that there was a normal arterial oxygen saturation during each observation.

Coronary Sinus Catheterization. The dog, lightly anesthetized, was placed on the operating-fluoroscopy table, and the neck shaved, scrubbed with sponges soaked in alcoholic zephiran solution, and draped. Clean but not aseptic technique was followed throughout, with all instruments, catheters and gloves soaked in 1:600 zephiran.

A branch of the external jugular vein was exposed as high as possible in the neck, and incised. A curved-tip intravenous catheter (sizes 7-8 F) was then inserted a short distance, and a slow, constant, saline drip, containing 10 mg. of heparin per liter, was connected to prevent clotting of blood in the catheter.

With the dog in the right anterior oblique position, the catheter was passed into the right auricle under fluoroscopic control. Once inside the superior vena cava, the curved tip was pointed anteromedially to avoid catching it on the ostium of the azygos vein or on the thickened ridge of muscle extending across the posterior auricular wall immediately above the foramen ovale. When the tip reached the inferior portion of the right auricle, near the tricuspid valve, it could be turned posteriorly and easily passed into the inferior vena cava (fig. 1). At this point, with the dog still in the right anterior oblique position, a triangular area of lung with the following boundaries was visible: 1. the anteromedial border of the inferior vena cava, 2. the posteroinferior cardiac border, and 3. the diaphragm. The coronary sinus ostium lies just anteromedial to the superior corner of this triangle, which marks the junction of inferior vena cava and right auricle, and which lies posteroinferior to the tricuspid valve. The catheter was now slowly withdrawn from the inferior cava to a point just inside the right auricle. If the tip was then turned and shifted slightly anteromedially, it pointed directly toward the coronary sinus ostium.



Fig. 1. Catheter in inferior cava (I.V.C.), illustrating lung triangle (L.T.) and relationship to coronary sinus (C.S.) and great cardiac vein (G.C.V.) in right anterior oblique view. Fig. 1 A. Catheter inserted 1 to 1-1/2 cm. into coronary sinus, position 1. Fig. 1 B. Catheter inserted 2 to 2-1/2 cm., just beyond the sharp bend in the sinus, position 2. Fig. 1 C. Catheter inserted into great cardiac vein, more than 3 cm. beyond the sinus ostium, position 3.



After one or two gentle thrusts, the catheter usually rounded a sharp initial bend (fig. 1A) and entered the sinus. The tip often passed further into the sinus, superiorly and to the left along the posterior auriculo-ventricular groove (fig. 1B) and sometimes beyond one or more delicate valves into the great cardiac vein (fig. 1C). Occasionally the ostium of the middle cardiac vein (fig. 5), located just inside the ostium of the coronary sinus (fig. 4), or more rarely, a posterior vein of the left ventricle (fig. 6), was accidentally catheterized. These veins pass along the posteroinferior septal surface toward the apex, anastomosing with each other and with the anterior descending branch of the great cardiac vein, as shown by diodrast injection in figure 6. In practice, however, only the proximal coronary sinus itself was catheterized (fig. 1A and 1B), as discussed below, because of the dangers and technical invalidity of coronary venous obstruction.

The advantage of the right anterior oblique position for visualization of the path of the catheter into the coronary sinus is illustrated in fig. 2B. In this view, a sharp bend was seen in the course of the catheter as it entered the sinus. The chief difference between the two pathways was the more posterior orientation of the coronary sinus, which became distinct as the animal was rotated to the oblique position. In this view, furthermore, the closest and most prominent landmark to the position of the coronary sinus ostium, namely the lung triangle and inferior vena cava, were well visualized. If the dog was turned too far toward the right lateral position (fig. 2A), the pathway into the coronary sinus became difficult to visualize because of a loss of three-dimensional perspective.

Coronary Venograms. Diodrast (3,5 diiodo-4-pyridone-N-acetic acid and diethanolamine) has been injected forcibly through the catheter against the stream of coronary sinus flow, and, as shown in fig. 4A and 6, the retrograde coronary venogram obtained. If the catheter obstructed the vein, as in fig. 5 and 6, diodrast could be easily made to fill most of the veins draining into the coronary sinus, due to the multiple veno-venous anastomoses. If the catheter lay freely in the coronary sinus, the diodrast was carried back along the shaft of the catheter in a visible stream, with the normal coronary sinus flow, until rapidly dispersed into the auricle at the coronary sinus ostium. The use of diodrast has helped to establish the orientation of the coronary sinus and coronary venous system as seen fluoroscopically, as well as the in vivo relationship of the coronary sinus ostium to the tricuspid valve (fig. 4A). Forcible injection is hazardous, however, since the dye may be forced into the tissues to cause local myocardial necrosis (fig. 5). Now that we are familiar with the fluoroscopic landmarks and the functional anatomy of the sinus venous system, we have discontinued diodrast injections.

Design of Coronary Sinus Catheters. The ordinary curved tip intravenous catheter presented the following drawbacks when used for catheterization of the coronary sinus: 1. Although fluid could always be freely injected through the catheter into the sinus, withdrawal of blood samples was often difficult or impossible. It is



Fig. 2

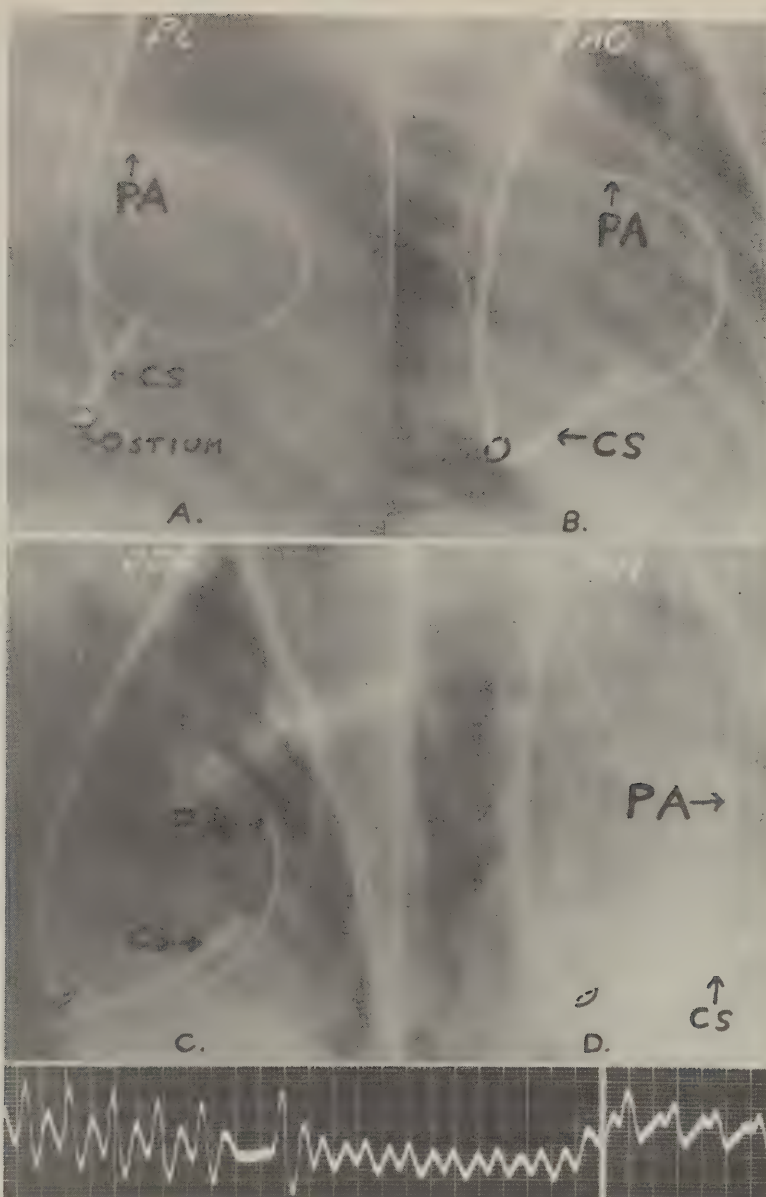


Fig. 3

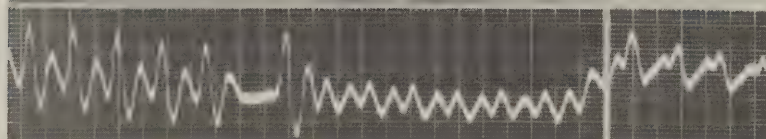


Fig. 2 A-D. Comparison of right lateral (RL), right anterior oblique (RAO), right posteroanterior (RPA) and postero-anterior (PA) in the same dog. Large catheter is inserted 4 to 5 cm. into coronary sinus, small catheter into pulmonary artery.

Fig. 3. Pulse pressure curves, slightly damped, during withdrawal of the catheter from the coronary sinus.

likely that the wall of the sinus, or a valve leaflet in the sinus or great cardiac vein, was drawn against the single catheter opening when suction was applied. This difficulty has been more serious, both in dogs and in man, when a small catheter (#6 or #7 F) has been used. . . 2. A small catheter (#6 or #7 F) would be most desirable for avoiding coronary venous obstruction. Such small catheters, however, were much harder to insert into the sinus than the larger sizes, because of the tendency for a small catheter to buckle in the auricle without passing around the sharp bend in the sinus. The ideal catheter, then, should have a small flexible tip for the part actually within the sinus, and a stiff, non-buckling shaft. This would give the best control on insertion, and the least obstruction and trauma to the coronary sinus. A larger, stiffer catheter may be actually less traumatic than a smaller one which is less easily controlled because of buckling (21).

A modified catheter has largely solved these difficulties. . The shaft tapers from a stiff #8F to a more flexible #6 or #7F between 4 and 6 cm. from the tip, and there are 2 additional side eyes 2 mm. back from the usual terminal eye. The side eyes tend to break any possible suction on a vessel wall or valve leaflet as a sample is withdrawn. The tendency to buckling is minimal with the larger shaft, while the smaller tip causes less trauma and obstruction within the coronary sinus. (These modified catheters are available on special order from the U. S. Catheter and Instrument Co., Glens Falls, N. Y.).

Catheterization of the Pulmonary Artery. For sampling mixed venous blood, a #6 or #7F curved tip intravenous catheter was inserted into the same vein used for the coronary sinus catheter. By clamping the opposite sides of the vein walls between the two catheters, retrograde bleeding could largely be prevented. A saline drip, containing 10 mg. of heparin per liter, was connected, and the catheter was then inserted into the pulmonary artery by the technique of Kinney, Haynes and Dexter (27). The tip was left far enough out into the lung field to be sure of its position, but not so far out, as Dexter and coworkers have shown can be done (21), as to draw fully oxygenated blood from the pulmonary capillaries or possible precapillary arterio-venous anastomoses.

Catheterization of the Femoral Artery. The femoral artery was exposed low in the femoral canal, and a #6 or #7 catheter inserted through a small incision in the artery, passed up into the aorta, and tied in place. The catheter was connected to a mercury manometer for pressure recordings except during sampling.

Sampling and Analytical Methods. Blood samples were drawn simultaneously from the femoral artery, pulmonary artery and coronary sinus through manifold systems (23).

Blood oxygen content was measured by the method of Roughton and Scholander (24), carbon dioxide content by that of Van Slyke and Neill (25). Expired air samples from the Douglas bag were analyzed in the Burrell analyzer, to determine total body oxygen consumption and carbon dioxide production. Total cardiac output was then calculated by the direct Fick method.

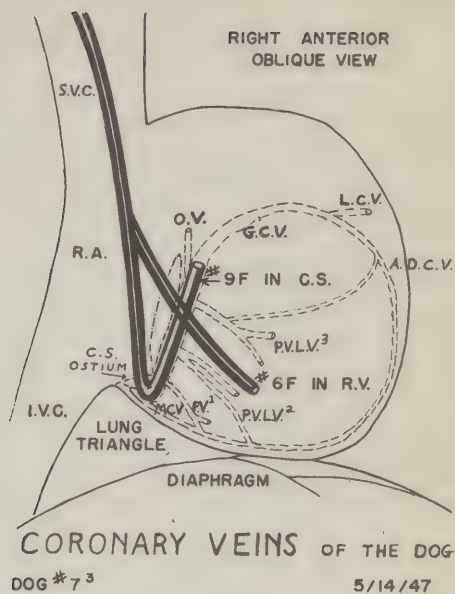


Fig. 4. Anatomy of the coronary sinus venous system in relation to a large #9 catheter, inserted 3 to 4 cm. into the coronary sinus (C.S.), with a small #6 catheter passing through the tricuspid valve into the right ventricle (R.V.).



Coronary Blood Flow was measured by the nitrous oxide method of Kety and Schmidt (12,23), as applied to the coronary circulation (14, 16). Glucose samples were analyzed by Nelson's method (26), using Somogyi's copper reagent (27), pyruvic acid by the method of Friedemann and Haugen (28) and lactic acid as outlined by Barker and Summerson (29).

## Results

The coronary sinus of 45 dogs, weighing 15-40 Kg., has been catheterized 68 times by this technique. There have been 3 failures, all in dogs under 15 kg. The procedure has permitted repeated observations of coronary blood flow and myocardial metabolism in the same dog as often as 7 times over a 4 month period, as illustrated by the protocol of Dog #7, fig. 4 and table 4. Fairly reproducible values for coronary blood flow and cardiac oxygen consumption were obtained, despite fluctuations and slight elevations of cardiac index, left ventricular work, mechanical efficiency, blood pressure, and particularly pulse rate above the basal level. The overall metabolic rate (oxygen consumption per sq. meter of body surface) was not significantly elevated.

A. Evidence of Successful Coronary Sinus Catheterization, (in addition to the typical fluoroscopic picture in the right anterior oblique view).

1. The dark color of the coronary sinus blood sample, corresponding to a very low oxygen saturation, clearly distinguished it even on inspection from other venous blood samples. The oxygen content of coronary venous blood averaged 4.1 volumes % with a mixed venous level of 13.0 and arterial level of 17.2. The mean coronary arteriovenous oxygen difference was thus approximately 3 times the total systemic A-V difference (table 1).

2. A typical pulse pressure pattern (fig. 3), was seen when the catheter was inserted well into the coronary sinus or great cardiac vein, correlated with a marked pulsation of the blood as withdrawn, or pulsation of the intravenous drip. This finding was considered to indicate at least partial coronary venous obstruction, confirmed by the elevated pulse pressure of 9-20 mm. Hg. compared with a right auricular pressure of 3-5 mm. Pulse pressures on insertion of a small catheter a similar distance, or on withdrawal of a larger catheter to position 1, just inside the coronary sinus ostium, were usually no higher than right auricular pressures (fig. 3B), with minimal visible evidence of pulsation in the intravenous drip.

B. Biochemical Characteristics of Coronary Venous Blood. Analysis of coronary venous blood also clearly distinguished it from mixed venous or arterial blood (table 1). No significant difference in these values was found when the catheter was inserted deeply into the sinus or great cardiac vein instead of only 1 to 2 cm. inside the ostium. The high coronary arteriovenous difference of oxygen, lactate, and pyruvate, considering the rate of coronary blood flow, indicate an extremely high rate of myocardial utilization of these



Fig. 5. Catheter obstructing the middle cardiac vein. The shadow of diodrast in the tissues illustrates the danger of forcible injections when the vein is obstructed.

Table 1. Biochemical Characteristics of Coronary Sinus Blood.

		Mixed Venous		Arterial		Coronary Sinus		Mean arteriovenous difference of simultaneous observations <sup>1</sup>	
		n	mean $\pm$ 6m	n	mean $\pm$ 6m	n	mean $\pm$ 6m	Systemic mean $\pm$ 6m	Coronary mean $\pm$ 6m
Oxygen, Vols. %	21		13.0 $\pm$ .50		17.2 $\pm$ .42		4.1 $\pm$ .19	+4.2 $\pm$ .36	+13.0 $\pm$ .54
Carbon dioxide, Vols. %	10		35.9 $\pm$ 1.20		31.8 $\pm$ 1.20		44.5 $\pm$ 1.26	-4.1 $\pm$ .19	-12.6 $\pm$ .71
Lactic acid, Mgm. %	9		13.5 $\pm$ 1.7		13.1 $\pm$ 1.7		7.7 $\pm$ 1.0	- .44 $\pm$ .26	+ 5.5 $\pm$ .86
Pyruvic acid, Mgm. %	14		1.93 $\pm$ .14		1.99 $\pm$ .17		1.07 $\pm$ .10	+ .07 $\pm$ .09	+ .93 $\pm$ .15
Glucose, Mgm. %	16		78.0 $\pm$ 3.3		79.3 $\pm$ 4.1		75.3 $\pm$ 3.6	+1.3 $\pm$ 2.5	+4.0 $\pm$ 2.6

<sup>1</sup>The difference between each pair of simultaneously drawn samples was obtained, and the average of these differences determined.



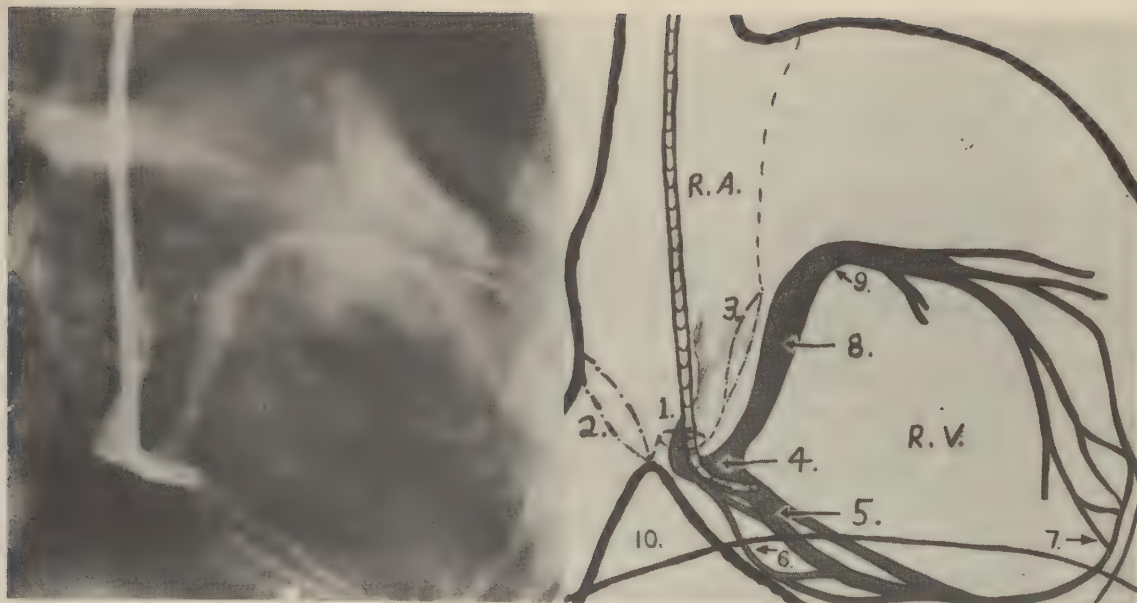


Fig. 6. Catheter obstructing the first posterior vein of the left ventricle. Diodrast injection outlines many of the veins draining into the coronary sinus through veno-venous anastomoses, 1. coronary sinus ostium; 2. inferior vena cava; 3. tricuspid valve; 4. coronary sinus, position 1; 5. first posterior vein of the left ventricle; 6. middle cardiac vein; 7. apical veno-venous anastomoses; 8. coronary sinus, position 3; 9. great cardiac vein, with valves illustrated; 10. lung triangle.

Table 2. Coronary Blood Flow with Catheter Tip Deep in Sinus

Date 1947	25 Jul.	11 Aug.	19 Aug.	27 Aug.	27 Aug.	3 Sept.	9 Sept.	Mean $\pm$ m
Dog No.	1 <sup>5</sup>	16 <sup>1</sup>	7 <sup>7</sup>	10 <sup>2</sup>	10 <sup>2</sup>	12 <sup>3</sup>	17 <sup>1</sup>	
Position of tip (fig. 1)	3	3	3	3	3	3	2	
Pulse rate/min.	100	136	160	120	136	140	148	134 $\pm$ 8.9
Mean arterial b.p., mm. Hg.	140	114	142	113	127	99	148	126 $\pm$ 6.9
Arterial O <sub>2</sub> content, vols. %	15.4	16.8	16.0	15.0	14.7	17.6	22.2	16.8 $\pm$ .99
Coronary O <sub>2</sub> content, vols. %	3.8	5.6	4.0	4.3	2.5	3.6	5.5	4.2 $\pm$ .4
Coronary flow cc./100 Gms./min.	70	77	82	80	70	69	67	73.6 $\pm$ 2.26
Oxygen consumption of heart cc./100 Gms./min.	8.1	8.6	9.8	8.6	8.5	9.6	10.2	9.1 $\pm$ .3

Table 3. Coronary Blood Flow with Catheter Tip near Ostium.

State	19	3	9	30	30	19	19	25	11	11	Mean	m
947	Aug.	Sept.	Sept.	Oct.	Oct.	Nov.	Nov.	Nov.	Dec.	Dec.		
Log No.	7 <sup>7</sup>	12 <sup>3</sup>	17 <sup>1</sup>	34 <sup>1</sup>	34 <sup>1</sup>	41 <sup>1</sup>	41 <sup>1</sup>	43 <sup>1</sup>	41 <sup>2</sup>	41 <sup>2</sup>		
Position of tip (fig. 1)	1	1	1	1	1	1	1	1	1	1		
Pulse rate/min.	156	140	148	160	160	140	148	142	130	125	145	±3.9
Mean arterial p., mm. Hg.	116	102	135	129	131	146	150	158	138	134	134	±5.1
Arterial O <sub>2</sub> content, vols. %	18.2	15.4	19.2	20.1	17.4	18.2	17.7	16.4	19.0	18.6	18.0	±.43
Coronary O <sub>2</sub> content, vols. %	3.7	2.5	3.7	1.9	2.6	4.0	5.0	4.2	4.6	3.9	3.6	±.31
Coronary flow c./100 Gms./min.	75	81	83	95	70	96	90	94	79	98	86.1	±3.10
Oxygen consumption of heart c./100 Gms./min.	10.9	10.4	12.9	17.3	10.4	13.6	11.4	11.5	10.4	14.4	12.3	±.7



metabolites in the normal intact lightly anesthetized dog. The rate of carbon dioxide production is correspondingly high. This confirms previous findings of many investigators who have used open chest and heart-lung preparations (30,33).

We have been unable to demonstrate significant removal of glucose by the heart by the present technique. The small mean arteriovenous difference of glucose, shown in table 1, could well be the result of sampling error.

Because of the interest in the constancy of the lactate/pyruvate ratio the relationship has been calculated on a small series of arterial bloods (n = 11), yielding a correlation coefficient of 0.88, and a mean L/P ratio of 77.

The cardiac lactic and pyruvic acid utilization alone may have accounted for as much as 50% of the total cardiac oxygen utilization in these experiments, assuming eventually complete oxidation of these metabolites to carbon dioxide and water.

C. The Anatomy of the Coronary Sinus Venous System. Twenty-eight anatomical dissections, correlated with diodrast injections in vivo, have helped to clarify the position of the catheter with relation to the coronary sinus venous system as seen fluoroscopically (figs. 4-6).

In summary, diodrast venograms and autopsy studies indicate that a non-occluding catheter, inserted only 1 to 1-1/2 cm. inside the auricular ostium of the coronary sinus, (position 1 or 2, fig. 1A or 1B) should collect representative samples of coronary venous blood draining almost entirely from the left ventricular myocardium. If this position of the catheter tip allowed withdrawal of blood from the middle cardiac vein, a significant proportion of the right ventricular myocardial flow might be obtained. Gregg has emphasized, however, that the anterior cardiac veins, which do not drain into the coronary sinus, are the major drainage channels of the right ventricle (13). The physiological role of the Thebesian veins is still disputed (10,11). Because of the sharp decrease in venous caliber at the opening of the great cardiac vein, however, varying degrees of venous obstruction may occur when the catheter is inserted too far, as already noted in vivo from pulse pressure recordings.

D. Hazards of Coronary Sinus Catheterization and Other Catheterization Procedures in Dogs. In 48 survival experiments, recovery was uniformly prompt, without any clinical complications attributable to catheterization. Postoperative care included one dose of penicillin in beeswax and oil, 300,000 units i.m., 0.4 Gms. of ferrous sulfate daily, and exercise with return to routine diet as early as 12 hours after the experiment.

Autopsy studies, however, often showed endocardial damage when the dogs were sacrificed following catheterization of not only the

coronary sinus but also the right auricle and pulmonary artery (34). A more controlled study is now in progress (35), but the findings incidental to the development of coronary sinus catheterization technique may be summarized as follows:

In a recent series of 28 dogs, autopsied within 10 days after catheterization, only 3 dogs were free of lesions at autopsy. Six had minimal lesions consisting of small subendocardial hemorrhages and tiny mural thrombi. The remainder had moderate to severe lesions of varying sizes, consisting of mural thrombi and subendocardial hemorrhages which sometimes extended well into the myocardium. These lesions occurred along the course of insertion of the catheter, including the tricuspid and pulmonary valves after catheterization of the pulmonary artery.

Six autopsies 3 to 6 weeks following catheterization showed only small patches of minimal subendocardial fibrosis in 3 cases. There was 1 case of marked fibrosis of the medial tricuspid valve leaflet, the significance of which was difficult to evaluate.

In these dogs, varying degrees of difficulty in catheterization were encountered. Catheters ranging from sizes 6 to 10 were used, usually #7, or #8. The catheter was left in place from 0 to 5 hours. Aseptic technique, omission of heparin administration, and use of a small catheter did not consistently eliminate the occurrence of endocardial lesions, whether the catheter was inserted into the coronary sinus, pulmonary artery, or only the right auricle.

This series was not well enough controlled to draw definite conclusions, but endocardial lesions were usually minimal when extreme care was taken to avoid trauma on insertion and when the catheter was left in place no more than 1-1/2 hours.

Deep catheterization of the coronary sinus, however, to position 3 or beyond (fig. 1C), often caused lesions peculiar to this procedure alone. There were 3 cases of gross myocardial hemorrhage in areas drained by the catheterized vein, particularly in the mitral valve and apical areas. In 2 additional cases of deep prolonged insertion, there was a well organized thrombus occluding the great cardiac vein. These lesions have been consistently avoided in 16 cases using definite precautions, including gentle insertion of a #7 catheter only 1 to 2 cm. inside the sinus.

### Discussion

Endocardial damage from catheterization of the heart by the present technique has not been reported previously in dogs or man, except for one case presented by Johnson (32). This patient had congenital heart disease with cyanosis and polycythemia, and at autopsy showed multiple thrombi along the course of insertion of the catheter, 1 month following the procedure. In several large series, however, including several thousand catheterizations by several investigators, and including numerous autopsied cases, there has been no apparent damage to the heart



from catheterization procedures in man (3,8,21). The fact that a catheter often causes endocardial lesions in dogs, but apparently not in man, may be related to differences in technique, or, more likely, to species differences in the response to injury. For example, the general tendency to blood clotting and postoperative intravascular thrombosis has been commonly observed to be greater in dogs than in man (34). However, thrombi in dogs were seldom present less than 12 hours after catheterization, and neither thrombi nor subendocardial hemorrhages were ever found in dogs sacrificed 3 weeks or more after catheterization, so that healing apparently occurred within 3 weeks. Lesions in man resulting from catheterization thus might not be found unless autopsy were performed within a comparable period. It seems unlikely that insertion of the catheter through the external jugular vein can be the determining factor, or that this approach is much more traumatic than the brachial approach used in man. Anesthesia may in some way influence the development of lesions. Tachycardia under nembutal anesthesia, and the poor fixation of the heart in the dog's mediastinum, may increase the friction between catheter and endocardium. The size of these hearts, however, (90-240 Gms.), is well within the range of the hearts of children and some adults.

In conclusion, deep insertion of a catheter 3 cm. or more into the coronary sinus or great cardiac veing in dogs often appears to be unduly traumatic, and undesirable because of coronary venous obstruction. This conclusion is based upon a correlation of anatomical and pathological studies with electrocardiographic and pressure recordings in vivo. A recent series of experiments (tables 1 and 3<sup>d</sup>) have yielded satisfactory coronary flow measurements with the catheter inserted only 1 to 1-1/2 cm. inside the sinus ostium, with minimal damage from the procedure, and with no evidence of contamination of blood samples with auricular blood. Therefore, definite precautions, including gentle insertion of a small catheter only a short distance inside the coronary sinus ostium, may well be advisable in applying this technique to similar studies in man, in view of the lesions produced in dogs. With these precautions in the dog, however, coronary sinus catheterization appears to be no more hazardous than catheterization of the pulmonary artery.\*

### Summary

1 A technique of coronary sinus catheterization in intact lightly anesthetized dogs has been presented. Modifications in the catheter design, and the optimal position of the dog for fluoroscopic control of insertion, have been discussed. The procedure has permitted

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\*The occurrence of endocardial lesions in dogs following catheterization of the pulmonary artery has been confirmed recently by Hellems, Haynes, Fanger and Dexter (33). The lesions were similar, but occurred with less frequency and severity than in the present series.



repeated observations of coronary blood flow and myocardial metabolism in the same animals over a period of months.

2. It has been found difficult to avoid endocardial damage entirely in dogs, following catheterization of not only the coronary sinus, but also the pulmonary artery. Peculiar to coronary sinus catheterization, however, were the coronary venous thromboses or myocardial hemorrhages which sometimes followed prolonged insertion of a catheter far into the coronary sinus or great cardiac vein. The elevated pulse pressure, 9 to 20 mm. Hg., found on deep insertion appeared to indicate a significant degree of sinus or coronary venous obstruction.

3. Coronary venous and myocardial damage were avoided by gentle insertion of a small catheter only 1 to 1-1/2 cm. inside the coronary sinus. In this position, there was no evident admixture of coronary blood samples with auricular blood, and evidence of trauma to the auricle or coronary sinus ostium was minimal. In this position also, the pulse pressure was not significantly higher than in the auricle, indicating that there was no significant coronary sinus obstruction by the catheter.

4. A high rate of cardiac utilization of oxygen, lactate and pyruvate with a correspondingly high production of carbon dioxide, was almost invariably found in the normal intact lightly anesthetized dog, but we were unable to demonstrate a significant utilization of glucose by the present technique.

5. Further possible applications of coronary sinus catheterization were illustrated, particularly the measurement of the oxygen consumption and mechanical efficiency of the heart.

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Table 4. Results of Repeated Coronary Sinus Catheterizations.

Dog #7, Male, 31 Kg.  
(See protocol, fig. 4)

Date 1947	17 June	8 July	29 July	19 Aug.	Average
Coronary Blood Flow <sup>1</sup> cc./100 Gms./min.	1) 78 2) 69	1) 91 2) 80	69	1) 82 2) 71	77
Cardiac O <sub>2</sub> Consumption cc./100 Gms./min.	1) 10.6 2) 9.7	1) 12.5 2) 11.0	10.4	1) 10.2 2) 10.3	10.7
Cardiac Index L/m. <sup>2</sup> /min.		2.9	2.7	4.7	3.4
Total Metabolic Rate cc. O <sub>2</sub> consumed/m <sup>2</sup> /min.		130	140	99	123
Mean Art. Blood Pres. mm. Hg.	1) 135 2) 147	1) 142 2) 146	142	1) 142 2) 115	138
Cardiac rate	1) 155 2) 160	1) 135 2) 130	140	1) 160 2) 156	148
Arterial O <sub>2</sub> Content Vols. %	1) 15.4 2) 16.3	1) 17.0 2) 16.6	18.5	1) 16.5 2) 18.2	16.9
Arterial O <sub>2</sub> Saturation		2) 93%	95%	2) 90%	93%
Coronary Sinus O <sub>2</sub> Vols. %	1) 2.0 2) 2.2	1) 3.3 2) 2.9	3.5	1) 4.1 2) 3.7	3.1
Left Ventricular Work <sup>2</sup> kg.-m./hr.		2) 336	306	2) 430	391
L. Ventricular Efficiency <sup>3</sup>		14%	13%	21%	16%

Heart Weight, 240 Gms.

<sup>1</sup>Measured over 5 minutes of nitrous oxide inhalation.

<sup>2</sup>Calculated as QR, neglecting the kinetic factor.

<sup>3</sup>Work/Oxygen consumption, both in caloric equivalents.

## CHANGES IN SKIN TEMPERATURE AND BLOOD FLOW

### OF HAND FOLLOWING INGESTION OF CERTAIN AMINO ACIDS

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Skin and rectal temperatures, oxygen consumption, and blood flow through the hands of 4 healthy men were studied before and after oral administration of various amino acids. At an environmental temperature of 24 C ingestion of glycine, in amounts of 1, 2, 3, and 4 grams/10 lbs body weight, resulted in average hand skin temperatures increases of 3.5 C°, 4.8 C°, 5.4 C°, and 7.7 C°, respectively, accompanied by similar, though less marked, increases in toe temperature and significant increases in blood flow through the hand as measured by venous occlusion plethysmography. Average maximum flows were 2.5 to 7 times the control flow. Increases in blood flow and hand skin temperature became apparent about 80 minutes after ingestion, being most marked approximately 180 minutes after ingestion. No significant changes in rectal temperature, or skin temperatures elsewhere were observed. At environmental temperatures of 18°C and 30°C, no significant changes in skin temperatures or peripheral blood flow occurred after glycine administration. At an environmental temperature of 24°C, ingestion of phenylalanine effected increases in hand and toe temperatures and blood flow through the hand similar to those observed with glycine; oral administration of histidine resulted in slight but significant increases in hand skin temperature and blood flow. Ingestion of glutamic acid, tyrosine, leucine, and methionine had no effect upon skin temperature or peripheral blood flow. Although 5 of the 7 amino acids caused definite increases in oxygen consumption, no consistent relationships between total oxygen consumption and skin temperature or peripheral blood flow were demonstrated.





## BIOLOGICAL STUDIES INVOLVING RADIOISOTOPES

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It is the purpose of the present paper to indicate a few of the applications of radioactive isotopes to biological problems in general and to illustrate in particular how current research in the field of metabolic exchange and material transfer has been aided by these technics.

As a working hypothesis, it is reasonable to assume that, if desired, radioactive isotopes may be employed in the majority of quantitative and qualitative laboratory procedures, and will enable measurements of the same order of precision as equivalent chemical procedures. The decision as to the practicality of such use will therefore rest upon the comparative efficiency of the particular determination by the two methods.

In biological chemistry, however, it is often desirable to know the nature of exchange of various chemical constituents normally present within the body. For this purpose only a labeling device of some sort will satisfy the need, and hence one must turn to some isotopic technic.

The labeling principle may utilize either isotopic mass differences as with the inert C<sup>13</sup>, N<sup>15</sup>, and O<sup>18</sup> or radioactivity, as with C<sup>14</sup>, S<sup>35</sup>, P<sup>32</sup> and a host of other radioisotopes.

The usual procedure in these tracer studies has been to obtain the radioisotope in a chemically pure state and to synthesize from it the desired compound. This requires due consideration of the many difficulties and associated hazards involved in handling the active sample.

With the development of the high intensity neutron sources of the uranium pile a substitute procedure has become feasible in a number of instances. This requires direct exposure of the inert chemical compound to neutron irradiation and permits utilization of the induced radioactivity for subsequent tracer studies. This procedure appears especially useful where relatively low activity is required, as for example, with in vitro experiments. It has the obvious advantage of eliminating much of the technical difficulty of radiochemical synthesis and moreover opens the stock commercial reagent and preparation supply to possible direct use in a radioactive form without necessity of synthesis by the investigator.

Turning to more specific applications of radioisotopes to problems of physiological interest, it is well to note that in addition to the chemical tracer functions indicated above, any isotope which emits gamma rays can be used as a topographical indicator of exchange over various body areas.

Thus with proper attention to shielding, a Geiger counter may be placed externally about the body and used either to survey distributional features of the administered isotope or to register in vivo its time course of appearance in the blood and tissues of an isolated region.

\ By the medium of gamma ray emission it has proved feasible to study the gross distribution of antimonial drugs in human patients (1) and similarly to follow the appearance of radioiodine in the thyroid (2) or radiosodium in the tissues of the extremities (3).

Beginning in 1941 this technic was exploited by the group at the Donner laboratory in Berkeley (Jones, Lawrence, Hamilton et al) to study the exchange of inert gases by the body. In this work the radioisotopes of argon, krypton and xenon were variously employed, being added in trace amounts to the breathing mixture of a closed respirometer system. Because of the ease with which an extremity may be shielded, the gas exchange of the hand, forearm or foot was principally studied. Various attempts were made to relate the measured rates of exchange of these regions, first, to the susceptibility of the individuals to high altitude decompression sickness or "bends", and later to the inert gas exchange of the body as a whole. While much was learned regarding the physiology of inert gas exchange and decompression sickness, a clear cut correlation between hand elimination rate and bends susceptibility has not been obtained. This lack of correlation is presumptive evidence for the intrinsic complexity of the basic processes involved. It is also indicative of the high order of variability which obtains when a response pattern rests upon several parameters.

Concurrently with the foregoing studies, the radioactive isotopes of the noble gases were also being employed at the Naval Medical Research Institute in Bethesda, Maryland. Early interest in this technic had been stimulated by Captain A. R. Behnke who was particularly interested in its applications to the physiology of deep sea diving. In order to extend these applications, however, it was apparent that formulation of a basic rationale was an essential prerequisite. This was subsequently developed in a series of papers (4, 5, 6, 7, 8) in which theoretical equations for the inert gas exchange of an isolated tissue region were formulated from a consideration of the basic parameters concerned.

The role of radioisotopes in this study was unique in that no other technic appeared capable of yielding a continuous in vivo assay of the state of the material balance within the isolated region. Thus from the results of Geiger counter measurements of the hand saturation curve for radiokrypton it became possible to confirm the predictions from theory regarding the shape and characteristics of the experimental curve.



Other experiments with radiokrypton were also conducted to determine how the hand saturation rate was influenced by changing various physiological parameters concerned with the exchange. For example, after a period of arterial occlusion lasting 10 minutes the hand saturation rate during the initial minutes after release was strikingly increased over the control level. This increase was in the direction expected since it evidently may be ascribed to the transient vascular flaccidity and increased blood flow rates which are known to follow a period of arterial occlusion.

It is of course well known that the amount of inert gas taken up at equilibrium will depend upon the size and composition of the body or region. Due to the greater solubility of most inert gases in fats as compared with water, differences in relative amounts of body fat constitute the most important single variable in deciding the equilibrium conditions. Determination of body composition thus constituted one of the important aspects of the inert gas exchange problem. Several methods have been employed to this end; of these the specific gravity procedures as developed and applied by Behnke and associates (9, 10) have been most useful. The precision of specific gravity methods, however, depends in part upon the use of average values for bone and muscle. It is therefore of interest to note that body composition can be measured by recourse to the differential oil/water solubilities of inert gases. A general method of this sort requires only that the equilibrium amounts absorbed be known for as many gases of different solubilities as there are solubility phases to be determined. For the human body treated as a two phase system this requires two such gases. One method has been proposed which utilizes radiokrypton and radioxenon for this purpose (11) but no experimental trials have been attempted. A special application of this general solubility method requires equilibrium data for only one gas, but must depend in addition upon some other independent measurement of one of the components. An example of one such independent method may again be drawn from recent work in which application of another radioisotope, namely  $H^3$  in the form of  $H_2O$ , has been of service in enabling accurate determinations of total body water in experimental animals and man (12).

### Summary

1. Radioisotopes may be serviceable in substitute procedures for a large number of routine laboratory technics now requiring physical and chemical assay methods.
2. Specific application of the radioactive isotopes of the rare gases to problems of decompression sickness has afforded technics suitable for in vivo study of inert gas exchange in isolated tissue regions. The results of such studies have confirmed a number of conclusions indicated from theoretical work.
3. Applications of the radiogases to determination of body composition have been indicated and the specific application of  $H^3$  to determination in vivo of body water is mentioned.



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# THE ACCELERATION OF BLOOD AND PLASMA COAGULATION

## BY LOW CONCENTRATIONS OF HEPARIN\*

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In the course of studies on the hemorrhagic manifestations of acute irradiation illness the occasional acceleration of the plasma coagulation rate associated with a low concentration of heparin was noted. This increased coagulation rate in the presence of a low concentration of heparin has previously been reported (1,3,4). However, Ferguson (2) was unable to confirm this finding. Further investigation of this possible relationship seemed indicated.

Heparin in 16 different concentrations, ranging from 0.0 mgm. to 0.015 mgm. per 0.3 cc. of plasma was added to plasma taken from a single goat. Each dilution differed by an increment of 0.001 mgm. per 0.3 cc. of plasma. One-stage prothrombin determinations (5) were run on these dilutions to show the relationship of a low concentration of heparin to changes in the plasma coagulation rate. The concentration of heparin capable of accelerating the coagulation rate was determined.

The effect of this concentration was studied in the plasma of 47 additional goats. One-stage prothrombin determinations were performed on each of the heparinized samples of plasma and were compared with samples of normal plasma from the same animals.

The acceleration of the plasma coagulation rate was also investigated in vivo in 4 goats following the intravenous administration of heparin. Prothrombin times (5), whole blood clotting times (6), heparin plasma concentrations (5), and hematocrit readings were determined at hourly intervals following injection.

The effect of increasing heparin concentrations in the plasma of a control goat is seen in table 1 and figure 1. At a concentration of 0.001 mgm. of heparin per 0.3 cc. of plasma an acceleration in the rate of plasma coagulation was observed. This concentration of heparin also produced an acceleration of the plasma coagulation rate in the 47 samples of heparinized goat plasma. The mean prothrombin time for the plasma containing 0.001 mgm. of heparin per 0.3 cc. of plasma was 0.7 seconds lower than the mean value of the control non-heparinized samples (table 2). A comparison of the data of the heparinized and

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\*The complete study is reported in report #2 of Project NM 007-039 subproject #3, Naval Medical Research Institute.

control plasma shows that the probability of these determinations occurring by chance is less than 0.01 and greater than 0.001 as calculated by Student's T Test (7).

After the establishment of the statistical validity of the in vitro studies the effects of the administration of intravenous heparin were investigated. In table 3 are found the changes observed in whole blood coagulation, one-stage prothrombin determinations, heparin blood titrations, and hematocrits following the intravenous injection of heparin in goats. Variations in the one stage prothrombin determinations were not as great in goats 103 and 129 as in goats 104 and 165. It was noted that the prothrombin times were shorter than the pre-injection determinations in all four animals. The same total dose of 100 mgm. of heparin was given to goats 104 and 165. However, the former animal weighed twice as much as goat 165 and the heparin levels of the smaller goat were approximately twice that of goat 165.

Figures 2 and 3 show the relationship of the whole blood coagulation measurement to blood heparin levels in the experimental animals. The blood coagulation determinations paralleled the changes in the heparin blood levels.

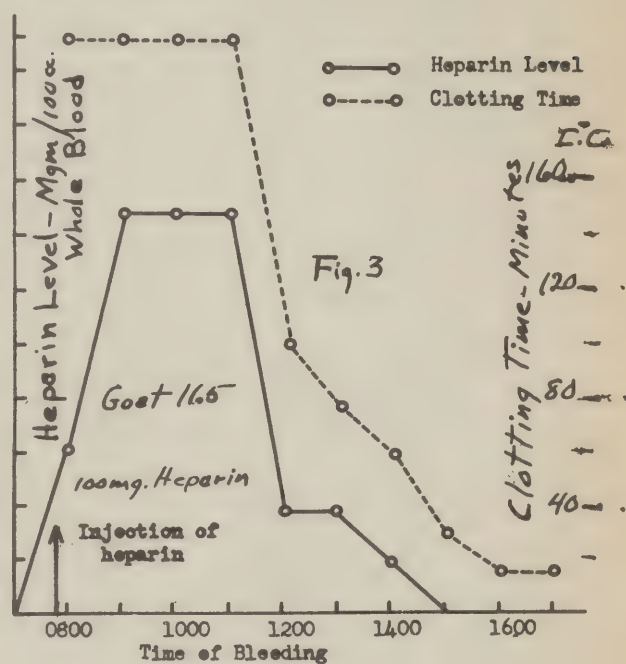
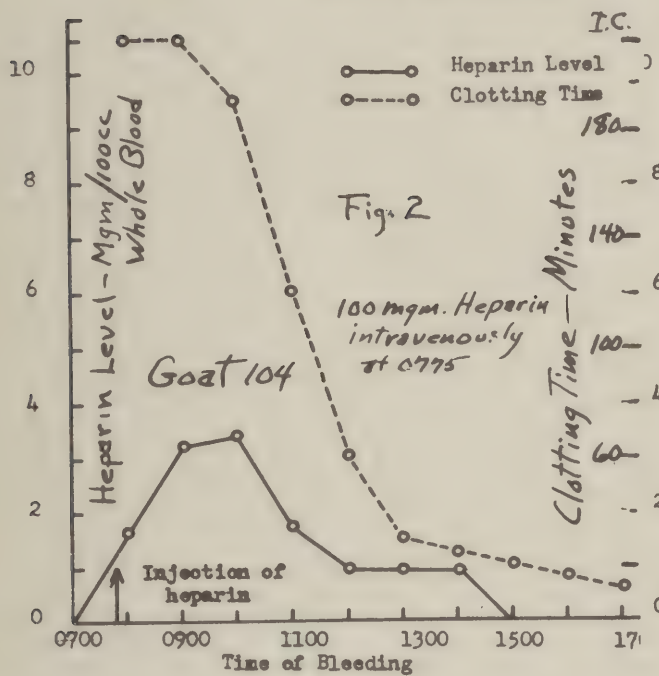
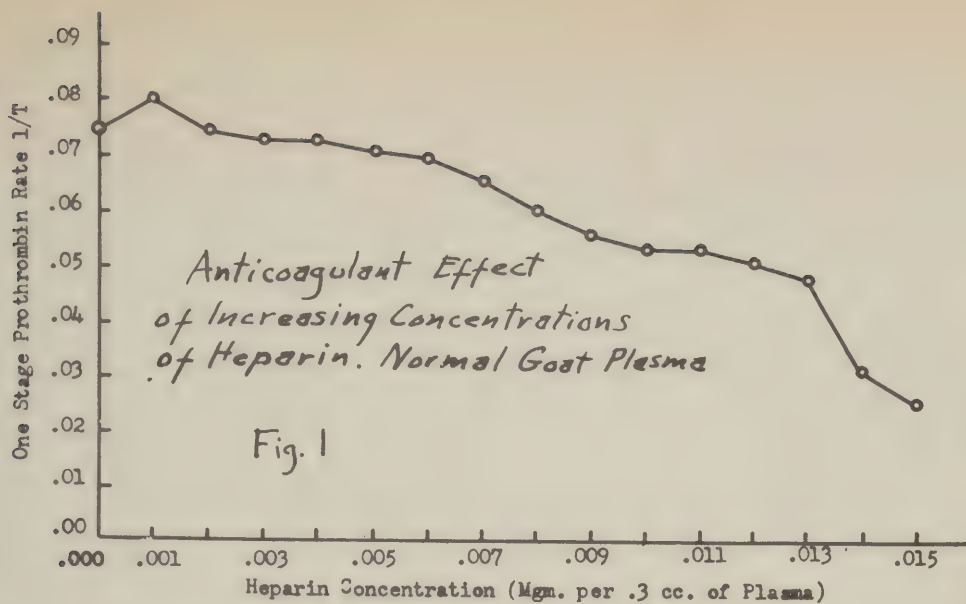
A comparison of the changes in the whole blood coagulation time for all animals given heparin is seen in figure 4. An apparent decrease below normal in the coagulation time was seen in goats 103, 129, and 104 approximately 6 to 8 hours following the injection of heparin. The duration of this effect was not ascertained.

The hematocrits of goats 104 and 165 exhibited a progressive fall after the injection of heparin. There was no evidence of hemolysis or external hemorrhage during this fall in the hematocrit.

The clinical importance of a possible acceleration in the rate of plasma coagulation in the presence of low concentrations of heparin has been emphasized by Welch and Faxon (8) and Allen, Linton, and Donaldson (9). These workers have reported cases in which thrombi and emboli occurred with increasing frequency within 24 hours after cessation of heparin therapy. It is possible that these phenomena are related to a transient hypercoagulability of blood induced by low concentrations of heparin. Warren (10) has also noted an acceleration in the blood coagulation rate immediately following exposure to ionizing radiation and others (11) have postulated that this may be due to the liberation of small amounts of a heparin-like substance titratable as early as 5 minutes after radiation.

The reasons for the variations of the one stage prothrombin times of goats 103 and 129 as compared to goats 104 and 165 are not apparent. The significance of the depression in the coagulation rate of animals 103, 129, and 104 below normal is emphasized when comparing the clotting times and heparin levels of these animals during identical sampling periods. The subnormal clotting values are obtained when the heparin level has fallen below titrable concentrations. The one stage prothrombin values follow the same trend as the whole blood clotting





times. The concentrations of heparin in the plasma and blood of goats 103 and 129 that accelerated plasma coagulation were four to thirty fold greater than those found by Tocantins (3) who reported an acceleration of coagulation of human whole blood with one to ten micrograms of heparin per 100 cc. of blood.

### Summary

1. An acceleration of the rate of plasma and blood coagulation by low concentrations of heparin has been demonstrated in vitro and in vivo.

2. Further work on the possible relationship of low blood concentrations of heparin to thrombotic and embolic phenomena is indicated.

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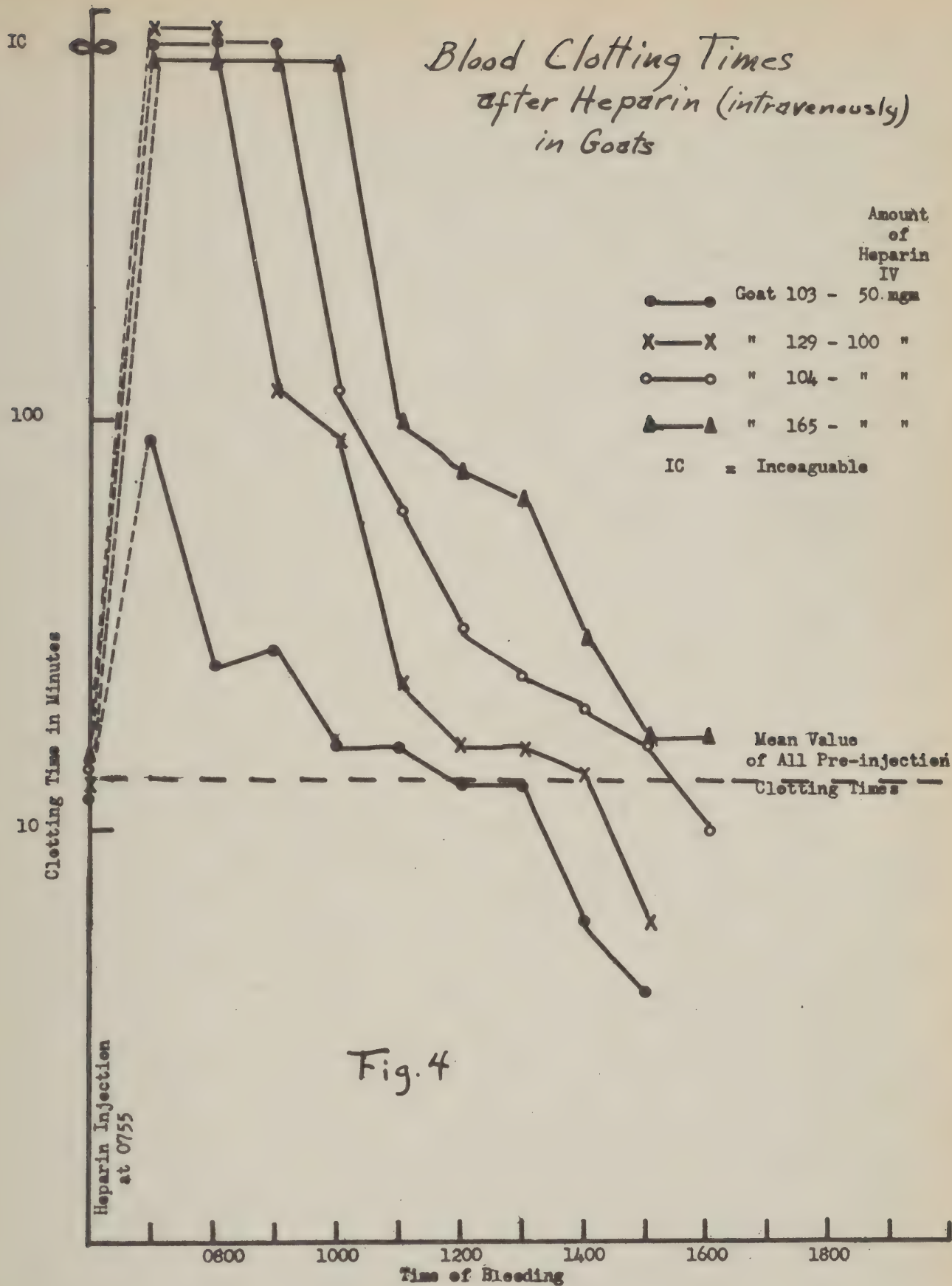


Fig. 4



Table 1.

HEPARIN DILUTIONS (Mgm. per .3 cc. of Plasma)

Trial No.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
	.000	.001	.002	.003	.004	.005	.006	.007	.008	.009	.010	.011	.012	.013	.014	.015
	One Stage Prothrombin Time (Seconds)															
1.	13.0	12.6	13.2	13.8	13.8	13.6	14.2	15.2	16.2	17.4	18.0	19.0	18.8	20.0	30.8	40.4
2.	13.0	12.4	14.2	13.8	13.8	14.0	14.0	15.2	16.0	17.0	18.2	18.2	19.2	20.8	30.0	<del>36.8</del>
3.	13.8	12.2	13.0	13.4	13.2	14.0	15.0	15.0	16.4	17.8	18.4	18.4	18.6	19.6	31.2	36.0
Ave.	13.3	12.4	13.4	13.6	13.6	13.9	14.1	15.1	16.2	17.4	18.2	18.2	18.9	20.1	30.7	38.4
Rate 1/T	.075	.080	.074	.073	.073	.071	.070	.066	.061	.057	.054	.054	.052	.049	.032	.026

Table Showing the Anticoagulant Effect of Increasing Concentrations of Heparin on Normal Goat Plasma.

Table 2. Statistical Summary of our one stage prothrombin time on heparinized and non-heparinized plasma of forty seven goats (23 males and 24 females).

	Coagulation Time in Seconds	
	Heparinized Plasma 0.001 mg. per 0.3 cc. Plasma	Non-heparinized Plasma
Range	8.2 - 13.2	9.7 - 14.9
Mean	10.9	11.6
Standard Deviation	1.2	1.1
Student's $t = 2.921$		0.01    P    0.001

Animal #	Blood Clot. Time in Minutes			One Stage Prothr. Time in Seconds			Mgm. of Heparin per 100 cc			Hematocrit in %				
	103	129	104	165	Plasma			Blood						
					103	129	104	165	103		129	104	165	
0700	12	13	14	14	12.2	12.4	12.4	13.9	0	0	0	0	40.5	30.0
0755					Heparin intravenously									
0800	90	IC	IC	IC	13.6	13.0	21.9	58.3	2.5	5.0	2.5	5.0	1.6	3.5
0900	25	IC	IC	IC	11.4	11.6	14.7	16.8	5.0	10.0	5.0	10.0	3.2	7.4
1000	28	120	IC	IC	14.0	12.4	12.7	24.5	5.0	2.5	5.0	10.0	3.4	7.4
1100	16	90	120	IC	11.0	11.2	12.4	13.7	2.5	1.3	2.5	10.0	1.7	7.4
1200	16	23	60	100	11.4	12.0	12.7	13.6	2.5	1.3	1.3	2.5	0.89	1.9
1300	13	16	31	77	10.2	12.8	12.9	13.4	2.5	1.3	1.3	2.5	0.33	1.9
1400	13	15	24	66	10.4	11.8	13.2	13.5	2.5	1.3	1.3	1.3	0.88	0.98
1500	6	14	20	30			11.4	12.2	=	-	0	0	0	30.0
1600	4	6	16	17			12.0	12.2	=	-	0	0	0	30.0
1700			10	17			11.6	12.2	=	-	0	0	0	30.0

The Relationship of Whole Blood Coagulation Time, One Stage Prothrombin Time, and the Hematocrit to Whole Blood and Plasma Heparin Levels, in Four Goats after Intravenously Injected Heparin.

Goat #	Weight	-56 Kg.	Infected on	9/15/47	at 0755	with 50	ngm
#103	Weight	-44 Kg.	"	9/15/47	"	"	100
#129	Weight	-44 Kg.	"	11/19/47	"	"	100
#104	Weight	-44 Kg.	"	11/19/47	"	"	100
#165	Weight	-25 kg.	"	11/19/47	"	"	100



## THE EFFECTS OF BURNS ON CARBOHYDRATE METABOLISM

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The work described in this paper is part of a study done in collaboration with Dr. James Walker, Jr., of the effects and treatment of severe flame burns, using the goat as the experimental animal. During the course of experiments on the treatment of burned goats with whole blood and plasma it was found that some of them were showing severe hypoglycemic reaction in contrast with the untreated burned animals, which normally were hyperglycemic. These results led to the following studies on blood sugar changes, intravenous glucose tolerance and on factors which are often related to changes in blood sugar concentration, including inorganic phosphorus, potassium and total carbon dioxide concentrations of the plasma and the plasma pH.

Methods. The goats used in these experiments were given third degree burns of the posterior portion of the animal, covering between 50 and 60% of the surface area. They were burned in a furnace with the flames of 12 oil burners, at a wall temperature of 1200 degrees C. The duration of exposure to flame was 12.5 seconds and was controlled automatically by a carriage which carried the animal into and out of the furnace. The area burned was circumscribed by glass cloth and the dividing line between the burned and unburned areas was sharp, with practically no first or second degree burns.

The goats were clipped, depilated and fasted for 24 hours before burning. They ate nothing after exposure and were given water by stomach tube. After burning, the animals were supported by slings in a yoke stall. Blood samples were drawn from the external jugular veins.

Blood for infusions was taken from healthy castrated male goats, citrate being used as the anticoagulant. The blood was controlled bacteriologically and the whole blood was used within 24 to 48 hours after collection. Plasma was kept for longer periods. A large number of cross-matchings were made on normal goats' blood, with no indications of blood groups or incompatibility. Gelatine solution (Knox "P-20", 6% gelatine in 0.9% sodium chloride solution, pH 7.2) was also used in some tests. The infusions were administered by gravity flow into the external jugular vein through a ureteral catheter inserted into the vein. Twenty-five to 30 ml. of blood or plasma per kg. or 20 ml. of gelatine per kg. was given at the following rates: blood, 4 ml. per minute; plasma, 6 ml. per minute and gelatine, 2 ml. per minute. The infusions were started within 15 minutes after burning.

Glucose was determined by Nelson's method (1). Blood drawn for the collection of plasma for inorganic phosphorus, potassium, carbon dioxide and pH determinations was centrifuged immediately after removal from the animal. For carbon dioxide and pH the plasma was collected and stored anaerobically. Potassium was determined with a flame photometer by the method of Hald (2). Inorganic phosphorus was analyzed as described by Fiske and Subbarow (3). The Van Slyke manometric blood gas analysis apparatus was used for the determination of total plasma carbon dioxide (4). The plasma pH was measured anaerobically at 29 degrees C. with a Cambridge glass electrode. Hematocrits were determined in Wintrobe tubes, spun at 3,000 R.P.M. for 30 minutes.

Intravenous glucose tolerance studies were done by administering 1.75 g. of glucose, in 40% solution, per kg. body weight via the external jugular vein. Blood samples for blood sugar determinations were taken before, and 15, 30, 45, 60, 90, 120, 180 and 240 minutes after glucose infusions. Glucose tolerance studies were made on the goats before burning for a control curve. Five to 6 hours after burning another glucose tolerance determination was begun.

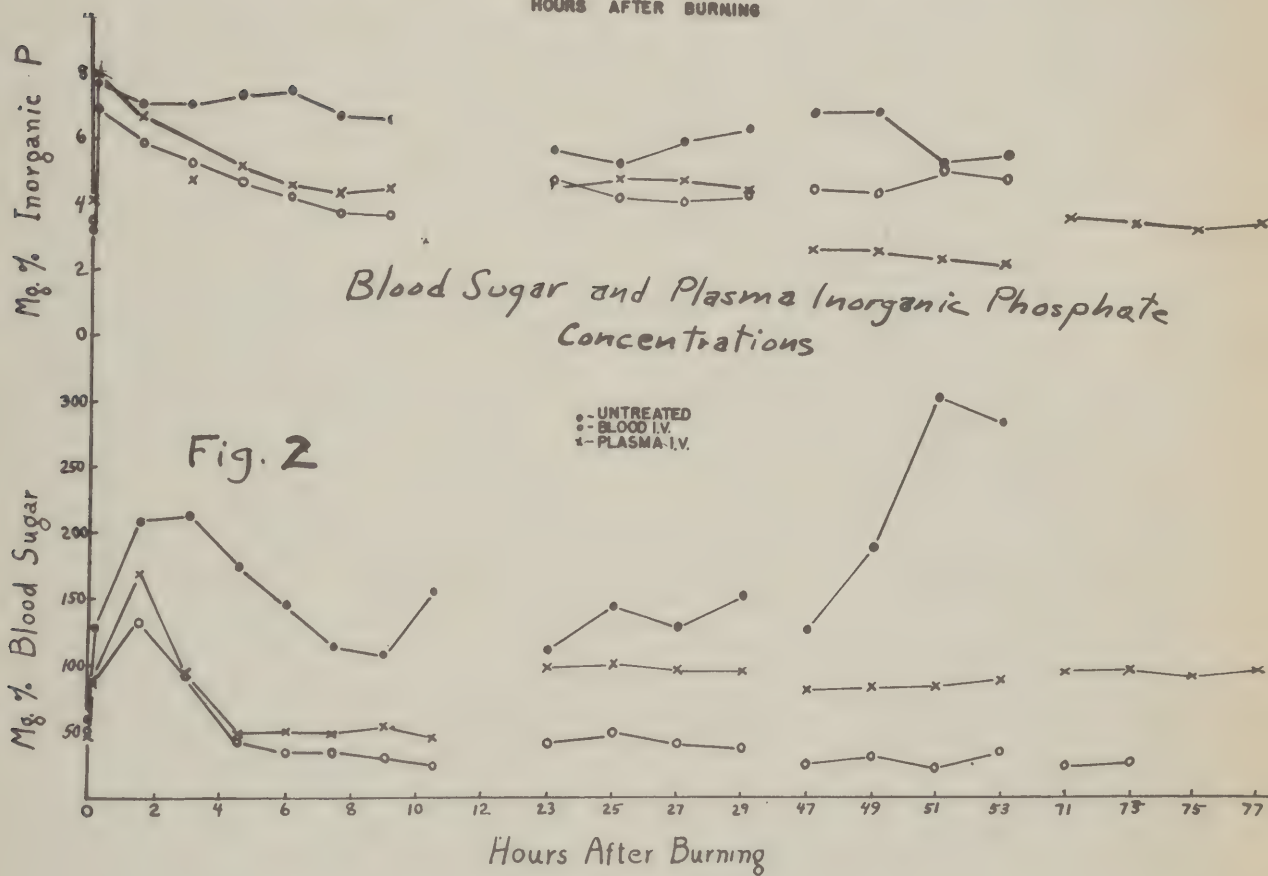
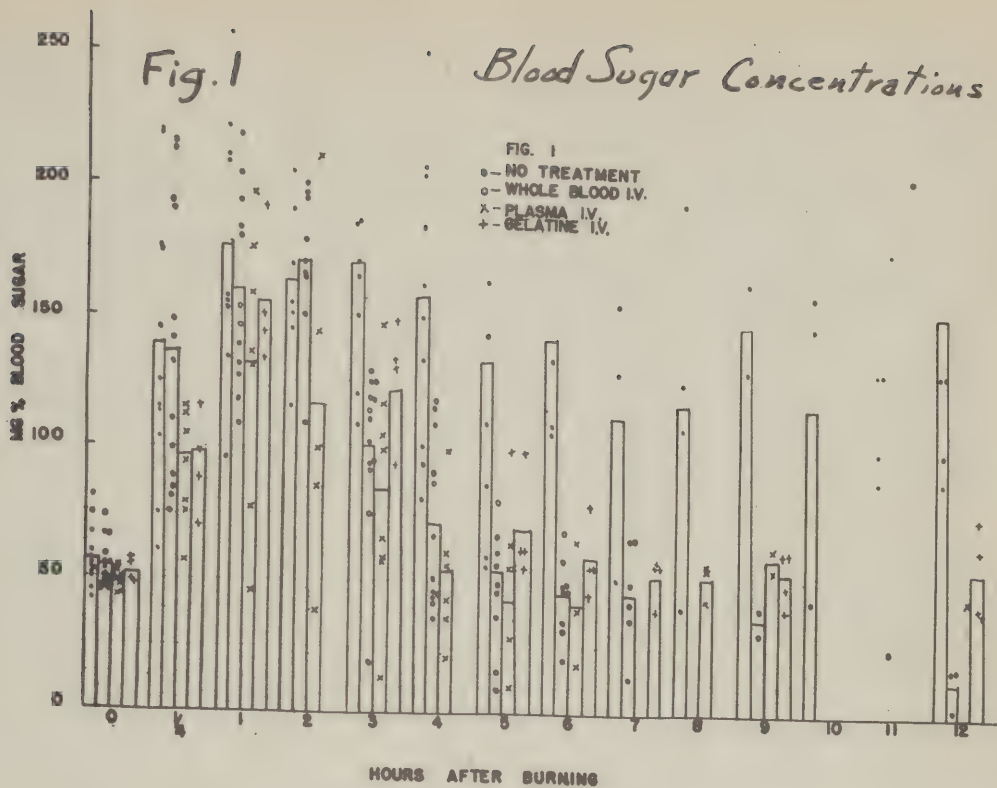
Results. a. Blood sugar. Flame burns caused a hyperglycemia in goats, which, in the untreated animal, often persisted until death. (fig. 1). In burned goats treated with blood or plasma the hyperglycemia disappeared more rapidly than in the untreated animals, and severe hypoglycemias, as low as 3 to 10 mg. %, often occurred. In gelatin-treated burned animals the hyperglycemia decreased more rapidly than in the untreated goats, but no cases of severe hypoglycemia were observed.

b. Factors often related to changes in blood sugar concentrations. Figures 2 and 3 show the results obtained from burned goats in the determination of blood sugar, plasma inorganic phosphorus, plasma potassium, plasma total carbon dioxide and hematocrit. The data are the averages of results from 3 each of untreated animals and those infused with whole blood, or plasma.

c. Plasma inorganic phosphorus. (fig.2). Immediately after burning the inorganic phosphorus rose to about twice its normal value. In untreated burned goats it remained elevated, while in the treated animals it decreased from the initial high concentration to normal or lower values. Preterminal plasma samples in both treated and untreated animals usually showed a high inorganic phosphorus concentration.

d. Plasma potassium (fig. 3). The potassium concentration rose immediately post-burning, but soon decreased, eventually to subnormal values. Treatment had no apparent effect on the plasma potassium changes after burning.

e. Total plasma CO<sub>2</sub> (fig. 3). Burning produced an immediate transitory fall in total plasma carbon dioxide. Subsequently the concentrations increased to normal or somewhat higher values. Treatment had no apparent effects.





f. Plasma pH. The plasma pH values paralleled the plasma total carbon dioxide concentrations.

g. Intravenous glucose tolerance. Figure 4 illustrates intravenous glucose tolerance curves on an untreated burned goat, a burned goat treated with whole blood and one treated with plasma. Also shown are sugar values of blood collected during the period, after the burn and before the tolerance test. The solid lines are the glucose tolerance curves on the burned animals. The broken line curves are normal preburn studies on the same animals, superimposed on the experimental curves by adjusting the initial blood sugars to the same value. Burned untreated goats had a decreased glucose tolerance at 5 hours post-burning. Burned goats treated with plasma or blood did not show this effect, but may even have an increased glucose tolerance, as can be seen in the curve for the blood treated burned goat.

Discussion. The initial reaction of the goat to the flame burn is in some ways similar to that caused by adrenalin administration. There is hyperglycemia, an initial rise in plasma potassium followed by a prolonged fall (5) and an initial alkali deficit (6). However, the hyperglycemia is usually more prolonged in the burned goat. Also, adrenalin is reported to cause an early lowering of plasma inorganic phosphorus followed by an increase (7). The plasma inorganic phosphorus of the burned animal increased initially, but this may have been caused by liberation of phosphorus from hemolyzed blood cells during the first stages of the burn. This hemolysis may also account, to some extent, for the initial increase in plasma potassium after burning, although the goat has a low erythrocyte potassium content as compared with the human. In general, it is probable that some of the initial effects reported in the above flame burn experiments are caused by increased secretion by the adrenal medulla.

The rapid disappearance of hyperglycemia and the frequent hypoglycemia found in the treated burned goat is not associated with changes in the acid-base balance. After the transitory acidosis immediately post-burning, both total plasma carbon dioxide and pH return to about the normal range, with no significant differences between treated and untreated animals.

Intravenous glucose tolerance studies made on treated and untreated animals 6 hours after burning show a decreased tolerance in the untreated goats and an increased or normal tolerance in the treated animals indicating a greater utilization of glucose by the treated goats. If this is a true increase in utilization, and is not a function of change in blood volume, extracellular space or blood flow, it would account in whole or in part for the differences in blood sugar in the treated and untreated burned goats.

Summary. 1. After being flame-burned, goats show a hyperglycemia, which decreases rapidly on treatment with blood or plasma. In many animals a severe hypoglycemia occurs after treatment.

FIG. 3

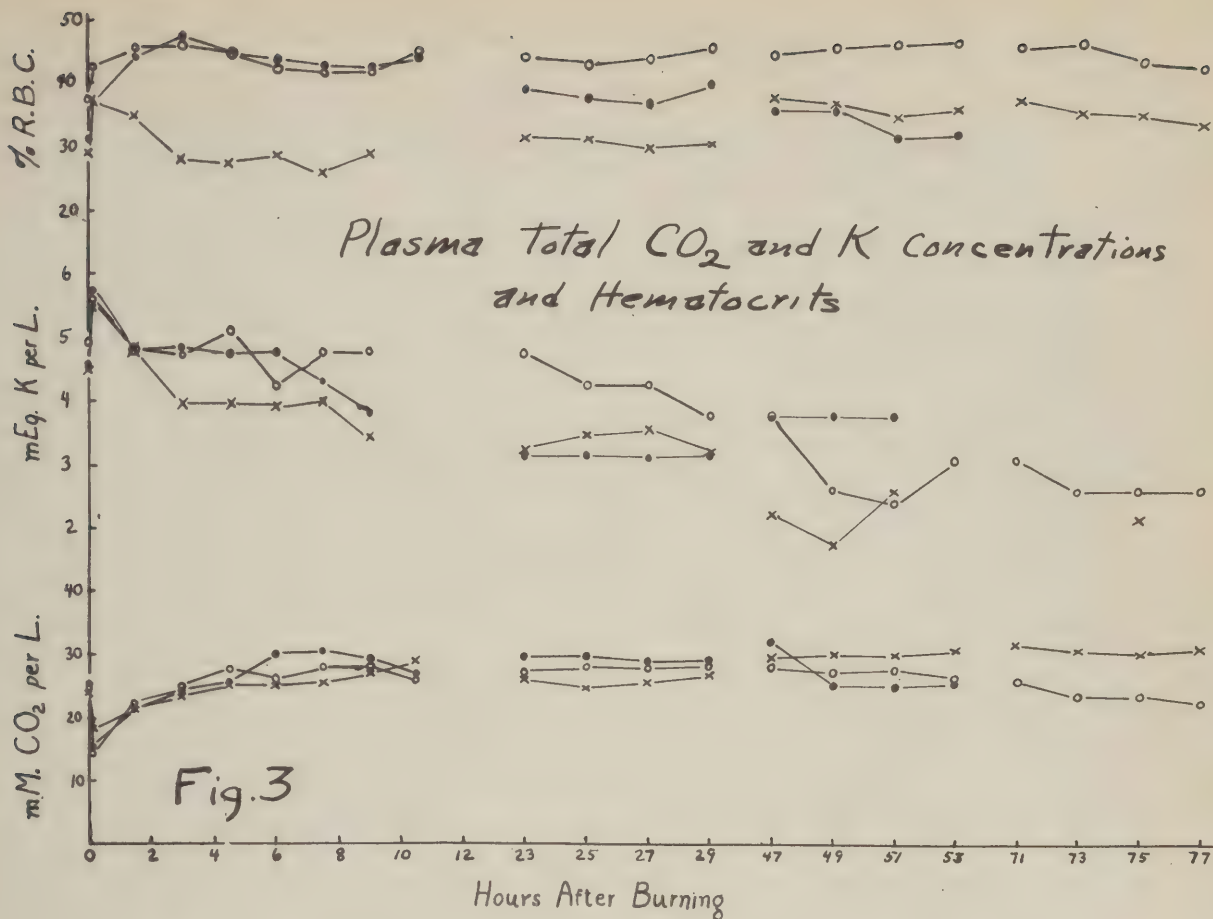
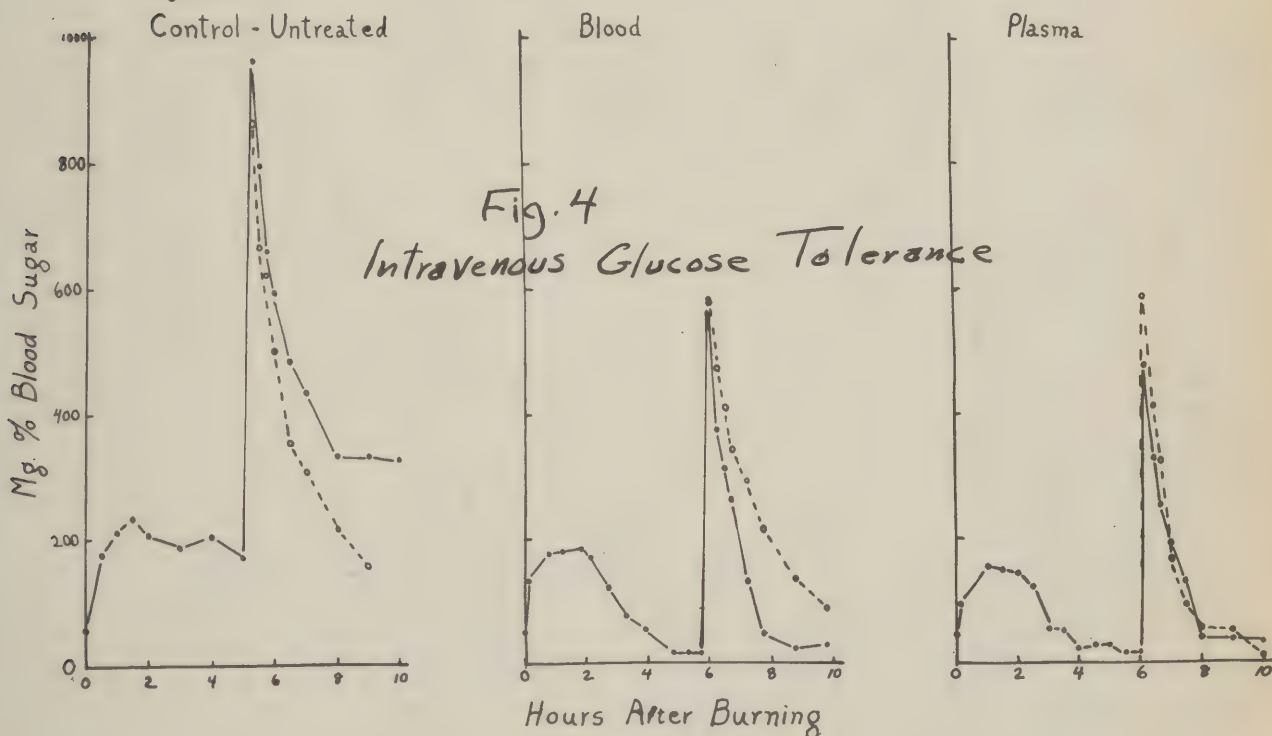


Fig. 4



2. During the first day after burning the plasma inorganic phosphorus concentration parallels, in a rough way, the blood sugar concentration, rising initially in all burned goats, remaining elevated in the untreated animals, and decreasing gradually in the treated goats.

3. Plasma potassium concentrations rise immediately after burning and then fall to normal or subnormal values.

4. There is no correlation between the changes in blood sugar and changes in the acid-base balance of the blood in the burned goats.

5. Intravenous glucose tolerance studies indicate a greater utilization of glucose in the treated burned animals than in the untreated.

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## FACTORS INFLUENCING ENDURANCE IN A WET-COLD ENVIRONMENT

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The object of this study was to determine and follow the physiological strains imposed by exposure for 48 hours to a wet-cold environment. Foxholes provided a convenient device for retaining men relatively immobile in this type of environment.

Eight enlisted men and 1 lieutenant of an infantry company were used as test subjects on 26 January 1947 on Adak Island. The foxholes were dug by the men 2 days before the test. The men were well rested and were devoid of any after-effects of alcoholic or other stimulants at the start of the test. Following preliminary measurements in a warm hut adjacent to the foxholes, 2 men were placed in each of 4 foxholes and 1 man in a fifth foxhole. The men were provided with the standard clothing for this environment plus the wet weather parka and trousers. Foxhole covers and sleeping bags were not permitted but the men were allowed to dig lateral tunnels in the foxholes for added protection if they so desired.

Food in the form of E ration was issued every 6 hours. Canned heat was issued at mealtime for warming the rations. Water and coffee were dispensed in measured quantities since information on diuresis was a part of the study.

The air temperature 3 feet above the ground was 28° to 30°F., and 30° to 33°F. at the bottom of the foxholes. During the first 14 hours the wind velocity averaged 30 m.p.h. During the last 10 minutes before observations were terminated, the wind velocity averaged 72 m.p.h. The men in the foxholes stated that the wind velocity had a negligible cooling effect but was annoying because dirt and snow blew into their faces and food. After 6 hours it began to snow and at the termination of the test the foxholes were half full of snow.

The men were under strict military orders to remain in their foxholes the full 48 hours. Their activities included digging, standing, sitting and lying. Only 1 man was able to sleep (during the last 2 hours of the test). Two others tried but were unsuccessful. Five men dug lateral tunnels into which they crawled. At intervals they came out "to stretch and take the stiffness out of their joints". Thus, cold was not the only stimulus for elevating heat production.

Five men were able to endure the foxhole conditions for only 16

hours (15.4 to 17.4 hrs.). They abandoned their foxholes and entered the hut contrary to orders. When this happened, the remaining 4 men were ordered to leave their foxholes (after 15.6 to 16.5 hrs.) and the test was terminated. On the following day all the men were individually interrogated. Those men who had not abandoned their foxholes made the following estimates as to their probable further endurance: 4 more hours for 1 man, 12 more for 2 men, and 1 man thought he could have stayed in the foxhole the full 48 hours. If one adds these personal estimates to the time these men actually stayed in the foxholes, the following endurance times can be stated: 6 men could endure only 15 to 20 hours; 2 men, 30 hours, and only 1 man 48 hours.

The 2 groups, the 5 men who abandoned their foxholes (Group 1) and the 4 men who did not (Group 2) cannot be differentiated on the basis of any of the physiological measurements that were made. Unfortunately, these measurements had to be terminated 1 to 3 hours (14 hrs. of exposure) before any of the men left their foxholes because the high wind velocity prevented all efforts to take measurements. Consequently, the data do not give information about the final critical moments, and it is possible that at the terminal stages of endurance physiological factors may have been the limiting ones.

Various body temperatures were measured thermoelectrically about once an hour. The rectal temperature did not fall but usually rose slightly. The skin of the abdomen and lower back always remained warm; in fact, the temperatures rose a few degrees after the wet weather parka was put on. The thigh skin cooled initially but warmed when the wet weather trousers were put on. The foot cooled slowly during the first 5 hours and then remained constant at about 60°F. The cheek, finger and great toe cooled quickly but the digital temperatures oscillated around a mean of about 55° to 60°F. No definite correlation can be made between any of these temperatures and endurance.

The heart rate was measured about once an hour by palpation of the radial artery. The man with the highest rates (fluctuating between 90 and 120 beats per minute) and the man with the lowest rates (fluctuating between 52 and 72 beats per minute) were in Group 1. The other 7 subjects had heart rates between these 2 extremes. No correlation with endurance can be made.

Kidney function, as measured by the diuretic effect of ingested water or coffee (500 cc.), also showed no correlation with endurance. All men had adequate fluid intake and their kidneys showed no inefficiency in eliminating or conserving water and solids.

The 2 groups did not differ significantly in average age (23 and 21 yrs.), average weight (152 and 154 lbs.), or average height (68.5 and 67.8 ins.).

Some differentiation can be made between the 2 groups on the basis of various psychophysiological factors. Apparently, hunger (stomach contractions) was inhibited in Group 1 since the average caloric intake of that group was less (1100 Cal.) than that for Group 2 (Group 1, 2170 to 3010 Cal.; Group 2, 3160 to 3930 Cal.). Body



temperatures were maintained by shivering in Group 1 and more by activity in Group 2, as deduced from hourly observations. Hourly reports indicated that the men of Group 1 felt subjectively colder than those of Group 2. Finally, 4 of the 5 men of Group 1 were definitely angered by their plight, whereas 3 of the 4 men in Group 2 were definitely not emotionally disturbed. No definite information was obtained from 1 man of each group but each showed no evidence of emotional disturbance.

It is of interest to note the factors inhibiting and those stimulating flight from the foxholes. Inhibiting factors were:

- a. The military order to stay in the foxhole.
- b. Social stimuli which encourage one to "take it" and discourage one from yielding to unpleasantness.
- c. Knowledge that the test would not last forever and that the observers would terminate it before deleterious effects supervened.
- d. Minor stimuli, such as those of personal relationship between observers and subjects, interest in aiding science.

Stimulating factors promoting flight from the foxholes were:

- a. Persistent cold.
- b. Wetness, disliked independent of its cooling effect.
- c. Stiffness from uncomfortable posture.
- d. Wind noise.
- e. Proximity of the warm hut (although 3 men said they would have left their foxholes even in its absence).
- f. The probability that disobedience would not meet with severe censure. (Three men said only definite knowledge of a severe court-martial penalty would have forced them to stay in the foxholes longer.)

These two antagonistic sets of stimuli were acting continuously. At first, the inhibiting set was prepotent but gradually in the course of 16 hours the second or excitatory set summated and, in Group 1, resulted in coordinated action. Undoubtedly, fatigue rendered the subjects more susceptible to the stimulating factors. How powerful this summation finally became may be gauged by the assertion of 4 men that even enemy fire would not have been an adequate stimulus to inhibit flight.

Some practical implications follow from the results of this test:



- a. Preselection. Men who cannot eat in the cold, who shiver rather than move around, who feel cold, and who become easily angered appear to be poor risks for protracted duty in a wet-cold environment.
- b. Prolongation of endurance. Measures for prolonging endurance should be those which reinforce the stimuli inhibiting flight or else narcotize the excitatory centers of the brain. Additional protection in the form of heat, clothing or shelter does not necessarily increase endurance. (Protection helps by removing the man from the environment.)
- c. The emergency mechanism of an individual may be hypersensitive, and may act to the disadvantage of the group without conferring real benefits to the man. Methods for diminishing the activity of the emergency mechanism deserve investigation.
- d. The subjects had had, prior to the test, an equal opportunity to acclimatize to the weather of Adak, because all of them had engaged in the same outdoor activities from the time of their arrival the preceding September. On the other hand, the fragmentary information obtained indicated that the men of Group 1 had had more experience in combat and in foxholes than the men of Group 2. The individual who was in active combat for the longest time (12 months in ETO) claimed that the test was one of his most harrowing experiences. It appears, therefore, that acclimatization (if it exists) and experience, either play a minor role in endurance or produce uneven effects among different men. The value of an acclimatization procedure remains to be demonstrated.

## THERMAL REGULATION DURING FEVER

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The gains and losses of heat by the body in fever were compared with those under normal conditions. The physiological mechanisms which influence these thermal flows were investigated under varied environmental conditions.

Ten young soldiers volunteered as subjects. Complete calorimetric studies were made on each in a single environment during one control and two fever runs. Values obtained in the control runs were compared with those obtained after intravenous injection of typhoid vaccine. Environmental temperatures ranged from 27° to 43°C and the humidity was low ( $P_{H_2O} \approx 7 - 12$  mm. Hg).

In the control experiments, heat transfer by all channels showed small fluctuations, but there was usually a small overall heat loss by the body, regardless of the environment.

Three physiological factors were found to play important roles in production of fever. Heat was conserved by reducing the sweat rate and the peripheral blood flow below normal values, and extra metabolic heat was produced in these experiments by the muscular activity of shaking chills.

In a cool environment, the first two factors were normally at nearly minimal levels, and restriction of them led to conservation of insignificant amounts of heat. The febrile rise was always induced by a shaking chill under this condition.

In a hot environment, on the other hand, the febrile rise was sometimes accomplished by restriction of sweating and peripheral blood flow alone, and there was no chill. In other experiments in hot environments the pyrogenic stimulus was apparently stronger and a chill occurred with the changes mentioned above. In the first case, low grade fevers were produced and, in the latter, high fevers occurred. It was a consistent finding that the first changes were those directed toward conserving heat and these were followed, in some cases, by increased heat production.

Slow defervescence may be accomplished passively in cool environments. Active cooling, the result of sweating and/or increased peripheral blood flow, is necessary to heat loss in hot environments, or to rapid heat loss in cool environments. Both mechanisms for active cooling were frequently employed simultaneously, but sometimes peripheral

flow was increased before sweating started. Metabolism was not reduced to subnormal levels during defervescence.

It is concluded that three physiological mechanisms are principally concerned in thermoregulation during fever; peripheral blood flow, sweat secretion, and muscular activity. These controls operate in certain patterns to produce fever, depending on the environment and the strength of the pyrogenic stimulus. The lysis of fever may be accomplished slowly by passive cooling in cool environments, but active cooling is always necessary in hot environments or for any rapid fall in body temperature.



# THE EFFECT OF AGE ON LETHALITY OF DI-ISOPROPYL FLUOROPHOSPHATE

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The role of acetylcholine in the central nervous system has as yet not been clearly established, although it is widely held that this substance participates in the transmission of the impulse in the peripheral nervous system. Due to the small quantities of acetylcholine present in the tissues and the great rapidity of its hydrolysis, direct study is well-nigh impossible (1). As has proved the case in analogous situations, the methods of enzyme chemistry have been helpful in the solution of this problem. The activity of acetylcholine is intimately bound up with the enzyme that hydrolyzes it, namely cholinesterase. Depression of cholinesterase permits accumulation of acetylcholine. On the other hand, increase in the activity of cholinesterase decreases acetylcholine. Since cholinesterase is a stable enzyme and can be determined readily by reliable methods, investigations of this enzyme yields significant information concerning acetylcholine.

In the past decade, study in this manner of the acetylcholine-cholinesterase relationship has advanced our knowledge of nervous mechanisms. Yet there is no unanimity of opinion, particularly in regard to the central nervous system. Some investigators in this field hold that the acetylcholine-cholinesterase relationship is crucial and indispensable for normal functioning of the brain, others believe that it is without such significance (2,3). The discovery of the action of di-isopropyl fluorophosphate (DFP), a toxic compound which either irreversibly inhibits or destroys cholinesterase (4) opened new pathways for the investigation of nervous mechanism both in the peripheral and central nervous system. Aside from its anticholinesterase effect, DFP has no action that has been conclusively demonstrated. This anticholinesterase activity, however, is powerful and specific and is, therefore, an important tool for the study of acetylcholine.

If two animal populations could be found that differed markedly in the cholinesterase concentration of the brain, the presence or absence of a variation in response to DFP might be indicative of the relative importance of this brain enzyme and its substrate, acetylcholine. Since Nachmansohn (5) has shown that the brain cholinesterase concentration of the adult rat is more than 4 times that of a newborn and also Welshe and Hyde (6) have demonstrated that the brain acetylcholine content of the adult rat is 4 times that of a newborn, our two populations were available.

The experiments were conducted in two parts. First the relative susceptibility of rats of various ages to DFP was studied. Secondly, the mechanism of the difference was investigated.

Methods. DFP was weighed out in amounts of 200 to 300 mgm and placed in hard glass ampules. Ampules were crushed in known volume of propylene glycol. The propylene glycol solution was then diluted with appropriate amounts of normal saline yielding solutions requiring the injection of 0.1 to 0.5 cc per animal.

Stock white rats of both sexes and of various ages were used in these experiments, the adults weighing up to 200 gm. Injections were made subcutaneously and intraperitoneally. In the case of the very young rats, arterial clamps were used to seal the needle puncture in order to prevent loss of even small amounts of fluid. For controls, newborn rats were injected with the vehicle used for the DFP.

To obtain information on the part played by the brain in the reaction to DFP, brain cholinesterase was determined on newborn and adult rats 30 minutes following the subcutaneous injection of 2.0 mgm/Kgm. DFP. The method was the manometric technic using the Warburg respirometer (5,7). A remarkable lowering of cholinesterase activity was found with this amount of DFP, so that the usual concentration of brain homogenate introduced into the respiratory chamber in dealing with the normal rat was inadequate to produce measurable quantities of CO<sub>2</sub>. Using varying amounts of the same brain homogenate it was observed that 250-400 mg of brain were necessary in each respiratory chamber. An example of this is illustrated in table 1. When such large amounts of brain are used in each flask the CO<sub>2</sub> and acid retention by protein becomes very important (8). The acid retention determined in the manner described by Umbreit (8) and Dixon (9) was found to alter the flask constant significantly both in the adult and in the newborn (table 2). On the other hand, CO<sub>2</sub> retention (8) was not of importance. Thus, in all determinations of DFP poisoned animals protein retention was taken into account.

Another possible correction to be considered is the DFP which might be retained in the brain tissue, and therefore might lower the actual cholinesterase activity during grinding. The DFP retention was checked in the manner described by Bullock et al (10) but no significant retention was disclosed (table 3).

Lastly, the brain cholinesterase on adult rats was determined 30 minutes following the subcutaneous injections of varying amounts of DFP. Clinical signs were carefully observed prior to sacrifice.

Results. The results of the subcutaneous injection of 175 rats are summarized in Chart I. In this diagram 5 hours survival are charted in order to avoid confusion with secondary effects such as inanition, but the results on a 48 hour basis are essentially similar. First, it may be noted that all the newborn controls survived. Second, that all injected newborns succumbed. Third, with advance of age, a generally increasing resistance up to a 73% survival in the adult rats.

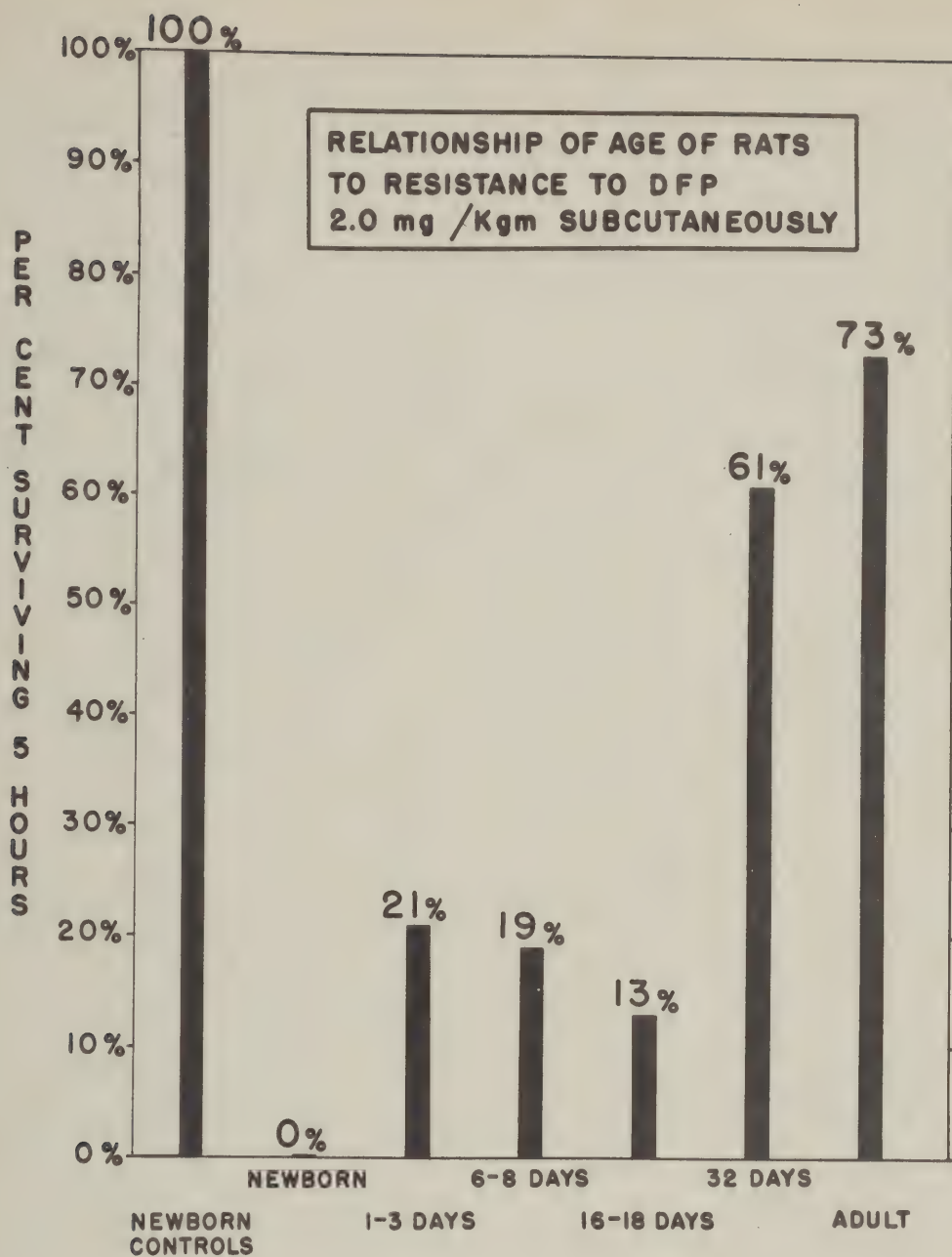




Table 1. Variation of CO<sub>2</sub> Production with Weight of Brain per Respiratory Chamber.

Mgm per flask	28	71	142	213	284	354	555
mm <sup>3</sup> CO <sub>2</sub> hr/100mgm	0	0	10	10	17	17	16

Table 2. Illustration: Change in Flask Constant Because of Acid Retention by Rat Brain.

Flask No.	9	10	11	12	13
Flask Constant	1.18	1.38	1.33	1.41	1.31
Flask Constant Corrected for 300 mgm Adult Brain	1.52	1.7	1.64	1.69	1.63
Flask Constant Corrected for 300 mgm Newborn Brain	1.39	1.64	1.65	1.59	1.63

Table 3. DFP Retention in Rat Brain

	Anticipated Value	Actual Value	% inhibition
Adult #1	376	361	4%
#2	194	185	5.4%
#3	196	201	---
Newborn #1	39	38	2.6%
#2	198	200	---
#3	103	143	---
#4	143	129	10%

Table 4. Brain Cholinesterase CO<sub>2</sub> Cu mm/100 mgm Brain/hr DFP  
2 mgm./Kgm Subcutaneous.

	Control	DFP	Remaining Activity
Adult	917 $\pm$ 168.6	27.3 $\pm$ 5.96	3%
Newborn	186 $\pm$ 45.12	8.1 $\pm$ 3.96	4%

Table 5. Relationship between Clinical Signs of DFP Toxicity and  
Brain Cholinesterase Activity.

No.	DFP mgm/Kgm. Subcu	Clinical Signs	Brain Cholinesterase CO /100 mgm Brain/hr.
6	0	None	917
3	0.5	Increased mouth movements, Exophthalmos	256-409
7	1.0	Slight trembling, fasciculation, moderate weakness of the hind limbs, some hyper-reactivity on tapping spine, "champing".	60-178
8	2.0	Marked salivation, "champing" pronounced weakness of all limbs, inability to crawl, exaggerated trembling, marked hyperreactivity on tapping spine.	20-39
6	3.0	Above signs in more severe form, tonic and clonic convulsions, either in extremis or dead.	11-16

The results of the intraperitoneal injections are, in general, in keeping with those obtained by subcutaneous injection.

Table 4 indicates brain cholinesterase activity in the adult and newborn normal rat and again after DFP. One is immediately struck by the marked lowering of brain cholinesterase activity by DFP--a decrease from 917 to 27 cu mm CO<sub>2</sub>/100 mgm tissue/hr in the adult and from 186 to 8 in the infant. Although the absolute amount of activity is significantly greater in the adult than the infant, the percent remaining is approximately the same--3% in the adult and 4% in the newborn.

The correlation between increasing severity of clinical signs and diminution of brain cholinesterase activity is brought out in table 5. The correlation observed in the adult rat is so consistent that it can scarcely be considered to be fortuitous. Furthermore, it appears that the critical level of brain cholinesterase activity permitting survival lies between 16 and 39 cu mm CO<sub>2</sub>/100 mg tissue/hr. It is of interest to note that the signs observed in the newborn poisoned with 2 mgm/Kgm DFP were, at first, "champing", then increased motion of the limbs, worm-like movements of the body, cyanosis, cessation of respiratory movements, and eventually, death.

Discussion. In order to explain the marked difference in susceptibility to DFP between the adult and newborn several possibilities must be entertained. First, one may consider the possibility of different rates of absorption from the subcutaneous tissue of the adult and the newborn. However, the essential similarities in the results using subcutaneous or the intraperitoneal route is evidence against any such explanation. Secondly, one may hold that the adult might be able to deviate and retain more DFP in the lipoid substance in the cerebral white matter which is much more abundant in the adult. However, investigation of the DFP retention has shown that such is not the case (table 3). By the same token, the failure to demonstrate significant CO<sub>2</sub> retention in the newborn brain indicates that the detoxification apparatus of the newborn is adequate for this dose of DFP and that the inability to destroy DFP does not explain the higher mortality in the newborn. Third, it is possible that the greater resistance of the adult is associated with the higher level of cholinesterase activities in the mature brain. The adult initially presents a higher level of cholinesterase activity than the newborn. Both groups suffer a percentage depression of equal amount after the injection of DFP. However, the adult is left with a higher absolute cholinesterase activity because of its higher starting value. Thus, the adult rat retains an amount which in most cases appears sufficient to sustain life. On the other hand, the newborn beginning with a less intense activity is left with a lower value. It is not known if the crucial level of brain cholinesterase activity is identical for the adult and the newborn. If one assumes they are approximately the same, then the longer survival in the adult is related to its higher level of brain cholinesterase. The possibility remains, however, that the infant requires a lesser activity than the adult to sustain life, a possibility that can be decided only by further work. This great sensitivity of the newborn is in striking contrast to its



greater resistance in other conditions such as anoxia (11), hypoglycemia (12), morphine poisoning (13), and alcohol toxicity (14). However, this is consistent with its greater sensitivity to barbiturates (15).

The greater vulnerability of the newborn rat to an anticholinesterase has many implications. It is well known that human infants are much more prone to convulsive episodes than adults. Since it has been suggested by many that convulsions are frequently associated with an excess of acetylcholine, (16, 17), this tendency of the young toward convulsions may be due to their greater sensitivity to various anticholinesterases both physiological and pathological. In the latter regard, one may recall that Gesell (18) has shown that  $\text{CO}_2$  is a most potent anticholinesterase.

Finally, we may discuss the role of the acetylcholine-cholinesterase relationship in the central nervous system. Investigators in this field have long regarded changes in cholinesterase as an indicator of reciprocal changes in acetylcholine, thus a diminution of cholinesterase activity is accompanied for a period of time by an excessive accumulation of acetylcholine. Further, the symptoms of acute DFP toxicity have been considered to be those of acetylcholine accumulation. Since it has been suggested in these experiments, that survival in the presence of an anticholinesterase is correlated with the maintenance of a high level of cholinesterase in the brain, these findings add further evidence that preservation of the balance in the acetylcholine-cholinesterase relationship is crucial for the normal functioning of the brain.

#### Summary

1. Newborn rats are much more sensitive to DFP than adults.
2. During the growth period, resistance to DFP increases with age until the rat is about 120 days old.
3. The impaired resistance of the newborn has been tentatively explained on the basis of lower cholinesterase concentration in the newborn brain, and consequently a lower reserve or safety factor.
4. A correlation between clinical signs and level of brain cholinesterase has been observed. The more severe symptoms are observed with greater depression of cholinesterase level.
5. These experiments point to a critical role for acetylcholine and cholinesterase in the central nervous system.

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## THE MECHANISM OF ACTION OF ANTICHOLINESTERASES

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The discovery of DFP and other synthetic phosphate esters added a new type of drugs to the pharmacological armamentarium - irreversible cholinesterase inhibitors lacking any independent pharmacological action. This is an important advance because it has been suggested that the older reversible esterase inhibitors of the physostigmine and neostigmine type have side actions which may interfere with the pure anticholinesterase effect of these agents. Some of these side actions may be of a paralytic nature; large doses of physostigmine or neostigmine depress the ganglia and have curare-like effects (Maurer; unpublished results). On the other hand, it has been suggested that neostigmine and other antiesterases behave like choline esters and produce direct stimulating effects on the receptor substance (1). Furthermore, there are indications that physostigmine and neostigmine potentiate acetylcholine effects independently of their inhibitory action on cholinesterase (1,2,3). It seems thus that the study of the mechanism of action of the different antiesterases must deal with two aspects of the problem, that of their protective action on acetylcholine, and that of their effect or effects independent of their anticholinesterase activity.

In recent years several methods have been devised to study these aspects of the problem. The deacetylase action on the esterase on acetylcholine may be measured by means of the titration of the acid or of the CO<sub>2</sub> liberated and the protective effect of antiesterases on acetylcholine thus be determined. The responses of an isolated skeletal muscle to acetylcholine and its subsequent relaxation with acetylcholine esterases have been standardized and used for the measurement of the antiesterase activities (4,5). While the first method is a typical in vitro method with its own advantages and drawbacks, the second method studies the response of an isolated organ, e.g., the striated muscle.

The third method utilizes the responsiveness to acetylcholine of the intact animal. It is well known that the inhibitors of the esterase potentiate both the physiological effects arising from the stimulation of the cholinergic nerves, and the pharmacological responses to injected acetylcholine. The latter comprise the so-called "nicotinic" and "muscarinic" effects (6,7). If the "muscarinic" effects of acetylcholine are studied in a non-atropinized animal in conjunction with the antiesterases, only small concentrations of the latter



can be used. Fatal effects of antiesterases in relatively small concentrations are due to bronchial spasm and cardiac arrest (8). On the other hand, if the "nicotinic" effects of acetylcholine are elicited in atropinized animals so that the muscarinic toxicity of the esterase inhibitors is counteracted by atropine, subsequent stimulation of the sympathetic autonomic ganglia or of the skeletal muscle can be safely potentiated by the use of anticholinesterases, some of which can now be employed over a wide range of concentrations up to 60 mgm. per kg. Moreover, these responses can be subjected to a quantitative analysis and studied in intact animals maintained in experimental conditions for prolonged periods of time. The latter method has been used in a standardized manner in this laboratory; the results obtained can be checked with in vitro results and the differences between the two sets of data pointed out.

### Methods

Dogs were anesthetized with nembutal (35 mgm. per kg. by vein) and atropinized with 10 mgm. per kg. of atropine sulfate by bein. The blood pressure was recorded from the cannulated carotid artery and the respiration recorded from a tracheal cannula connected with a tambour. Throughout the experiments standard test doses of acetylcholine (e.g. 0.025, 0.05 and 0.10 mgm. per kg.) were administered by vein. Ultimately plots were constructed to relate the concentrations of the anticholinesterases to the hypertensive effect of the test doses of acetylcholine (figures 1-5). Special attention was paid to the character of the curves thus obtained, to the slope of the onset of action, etc.

Possible objections to this method were investigated. It was important, for instance, to ascertain that the atropinization does not influence the ganglionic response to acetylcholine. It has been, indeed, pointed out by Marrazzi and Jervik (9) that in their preparation of the inferior mesenteric sympathetic ganglion of the cat atropine may inhibit synaptic transmission. In our experiments, doses up to 15 mgm. per kg. of atropine did not diminish the "nicotinic" response of the ganglion to acetylcholine or to nicotine itself. It was also necessary to determine whether the response of the ganglion was not affected by its activity due to the frequent administration of test doses of acetylcholine. The fact is that the "nicotinic" effect remained fairly constant over prolonged periods of time (4 hours or more) in spite of more than 50 acetylcholine injections.

### Results

Generally, all the curves obtained for the relationship between the concentrations of different antiesterases and the pressor response to the test doses of acetylcholine were characterized by a maximum (fig. 1-5) which is understood as corresponding to that level of the anticholinesterase which inhibits completely the activity of cholinesterase (100% inactivation point; see Discussion). From the curve the concentrations of the inhibitor required to produce a 50% inhibition

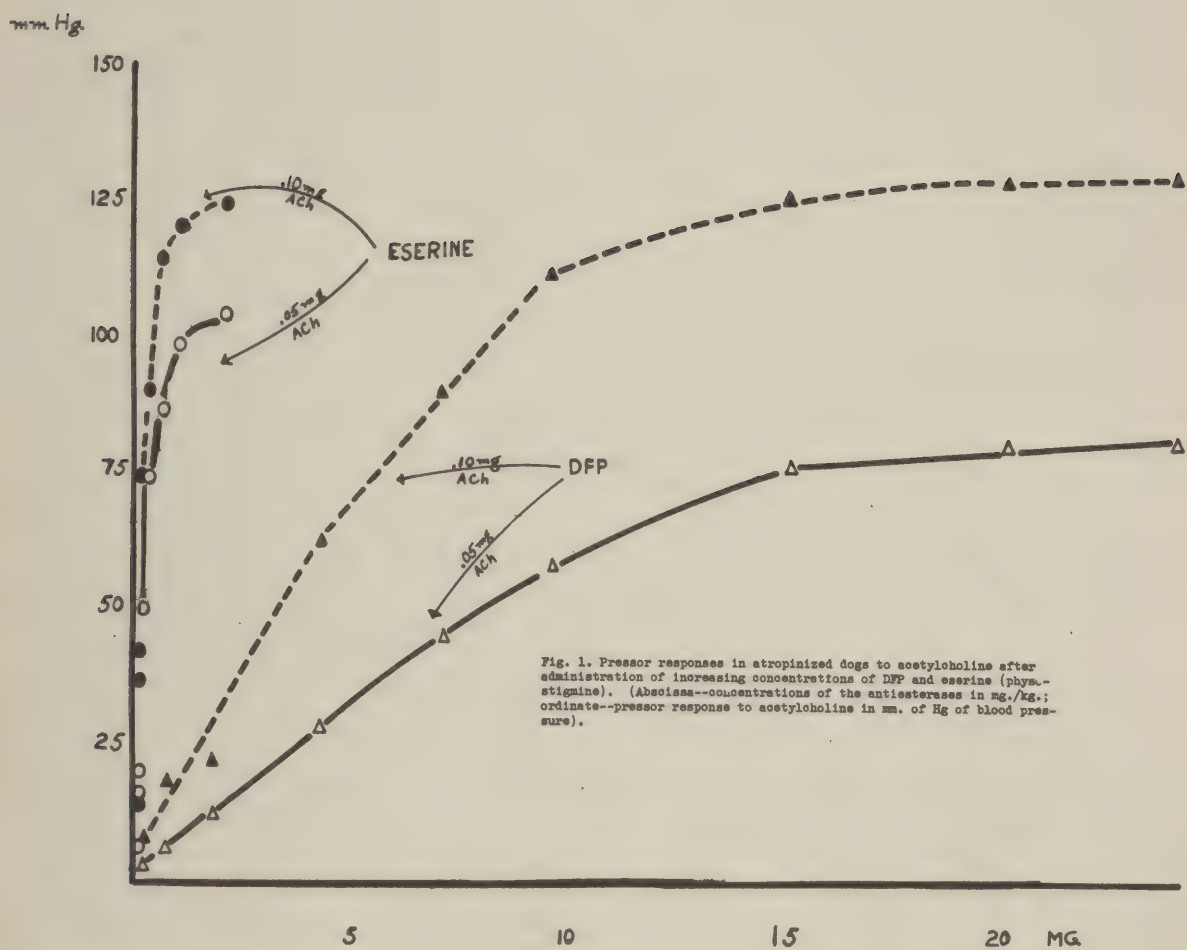


Fig. 1. Pressor responses in atropinized dogs to acetylcholine after administration of increasing concentrations of DFP and eserine (physostigmine). (Abscissa--concentrations of the anticholinesterases in mg./kg.; ordinate--pressor response to acetylcholine in mm. of Hg of blood pressure).

of cholinesterase can be read also (table 1). One fact has to be emphasized, namely that the 50 or 100% inhibition points correspond to the magnitude of concentrations of the inhibitor capable of effecting the same degree of inhibition in vitro (table 1). Actually, the concentrations required for the 100% inhibitions are frequently lower when obtained by the method described here than when recorded by the in vitro techniques, which seems to indicate a high degree of sensitivity and specificity of the in vivo method. Small concentrations of some antiesterases can produce a recordable result in terms of their potentiation of the effect of the test dose of acetylcholine (table 2) and with high concentrations of the antiesterases, as small a dose of acetylcholine as 0.8 gammas per kg. is sufficient to elicit measurable pressor responses.<sup>1</sup> It has to be pointed out, however, that the character of the curves is such that the 50% inhibition point read from them may or may not correspond to the in vitro results.

TABLE 1. Sensitivity of acetylcholinesterase to various anti-esterases.

	DFP	HETP	Eserine	TEP
Molar concentrations of inhibitor required to produce 50% inhibition of cholinesterase activity.				
Roach cord	3.0 10 <sup>-5</sup>	4.0 10 <sup>-6</sup>	1.0 10 <sup>-8</sup>	_____
Human serum	5.0 10 <sup>-7</sup>	_____	5.0 10 <sup>-6</sup>	_____
Horse serum	1.3 10 <sup>-8</sup>	_____	_____	_____
Cockroach serum	_____	1.0 10 <sup>-7</sup>	_____	_____
Rat brain	6.3 10 <sup>-8</sup>	1.6 10 <sup>-8</sup>	_____	4.0 10 <sup>-9</sup>
Dog (intact)	5.0 10 <sup>-9</sup>	4.0 10 <sup>-10</sup>	9.5 10 <sup>-11</sup>	8.5 10 <sup>-11</sup>

Molar concentrations of the inhibitor required to produce 100% inhibition of cholinesterase activity.

Rat serum	_____	2.0 10 <sup>-9</sup>	_____	_____
Dog (intact)	7.5 10 <sup>-8</sup>	1.0 10 <sup>-9</sup>	7.9 10 <sup>-10</sup>	3.3 10 <sup>-10</sup>

<sup>1</sup>This figure compares favorably with that of Dale (10) of 2 gammas for the minimum amount of acetylcholine necessary to stimulate on close intra-arterial injection the striated muscle of the frog, and with the minimal amounts previously estimated to be necessary to produce sympathetic ganglionic effects.



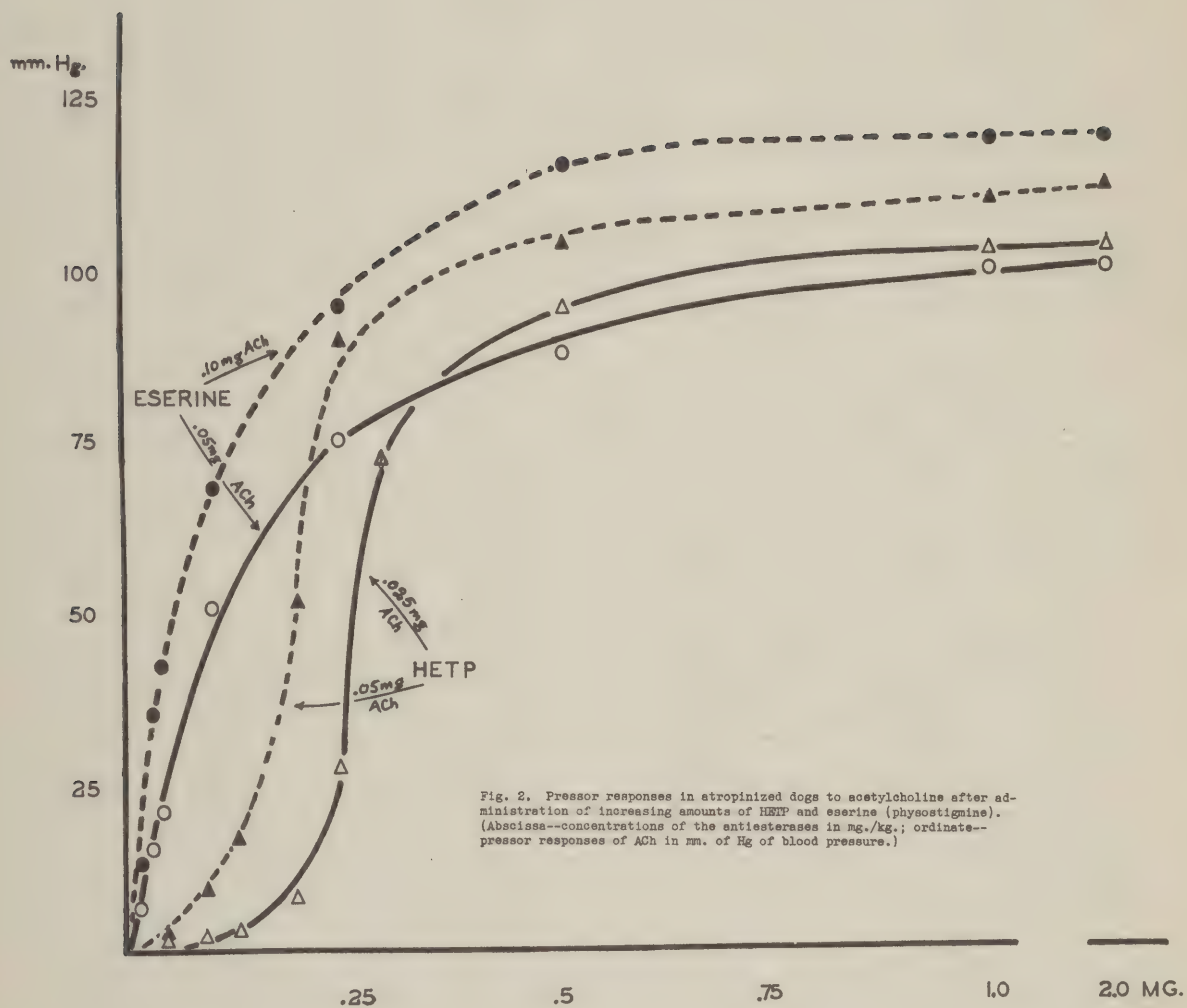


Fig. 2. Pressor responses in atropinized dogs to acetylcholine after administration of increasing amounts of HETP and eserine (physostigmine). (Abscissa--concentrations of the antilesterases in mg./kg.; ordinate--pressor responses of ACh in mm. of Hg of blood pressure.)

TABLE 2. Effective critical doses of antiesterases determined from acetylcholine pressor responses in atropinized dogs.

	Physostigmine	Neostigmine	DFP	HETP	TEP
Minimal effective dose, mgm. per kg.	0.040	0.010	0.50	0.050	0.01
Optimal effective dose, mgm. per kg.	0.750	1.000	15.000	0.750	0.10

Except for the appearance of the 100% inactivation point which characterizes all these curves, the activities of the antiesterases and the properties of the pertinent curves vary widely:

a. The minimal effective dose of the different antiesterases, that is the concentration of the inhibitors at which the effect of the test doses of acetylcholine is potentiated for the first time, varies from one agent to another, as shown in table 2.

b. In general, the same range of differences characterizes the optimal effective doses of the antiesterases. Concentrations of the inhibitors higher than shown in pertinent columns of the tables 1 and 2 do not further increase the pressor effect of the test doses of acetylcholine, with exceptions noted below. Both the minimal and optimal effective doses of TEP furnish proof that this compound is the most potent esterase studied to date (11).

c. The above differences in potency between the antiesterases as well as probably other differences between these agents are reflected by the dissimilar character of the curves relating the activity and the concentrations of these agents.

(1). Physostigmine (fig. 1-3); TEP (fig. 3,4); and neostigmine (fig. 5) seem to have a biphasic effect on the "nicotinic" action of acetylcholine, the latter showing a double peak in plotting the size of the pressor effect. The two peaks are separated by a series of lower hypertensive readings.<sup>2</sup> The second peak which seems to be generally, but not necessarily, higher than the first occurs with very high concentrations of antiesterases - 20 mg./kg. for TEP and physostigmine, and 5 to 8 mg./kg. for neostigmine. Tables 1 and 2 show that the concentrations which inactivate esterase completely in the in vitro experiments and elicit the first peak response in the intact dog are but fractions (from 1/5 for neostigmine to 1/200 for TEP) of the

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<sup>2</sup>Physostigmine at certain dosage levels also diminishes the pressor activity of nicotine. Higher concentrations of physostigmine restore the original pressor effects. Since nicotine acts presumably independently of cholinesterase, sui generis ganglionic damage by physostigmine must be involved.

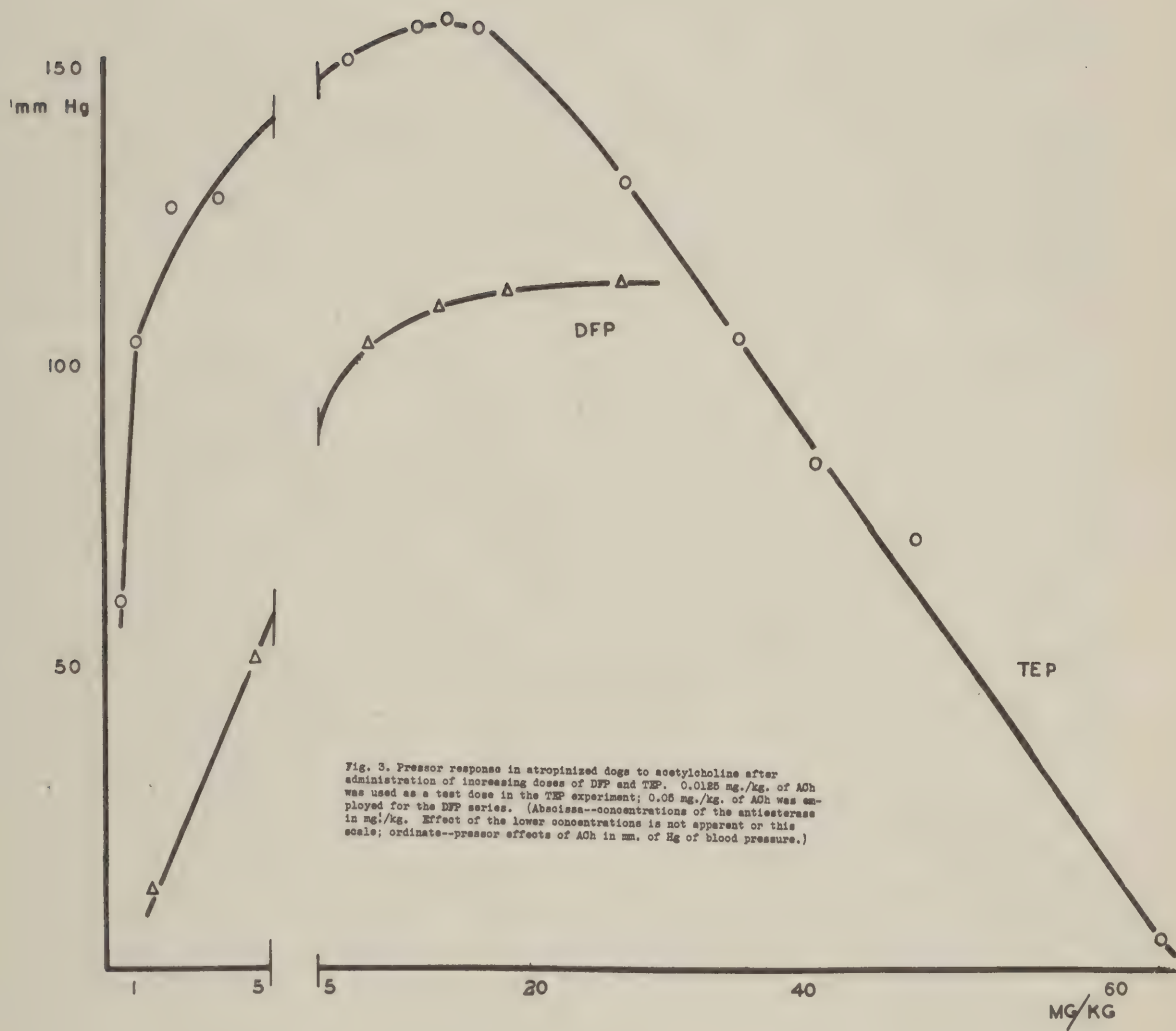


Fig. 3. Pressor response in atropinized dogs to acetylcholine after administration of increasing doses of DFP and TEP. 0.0125 mg./kg. of ACh was used as a test dose in the TEP experiment; 0.05 mg./kg. of ACh was employed for the DFP series. (Abscissa--concentrations of the anticholinesterase in mg./kg. Effect of the lower concentrations is not apparent on this scale; ordinate--pressor effects of ACh in mm. of Hg of blood pressure.)

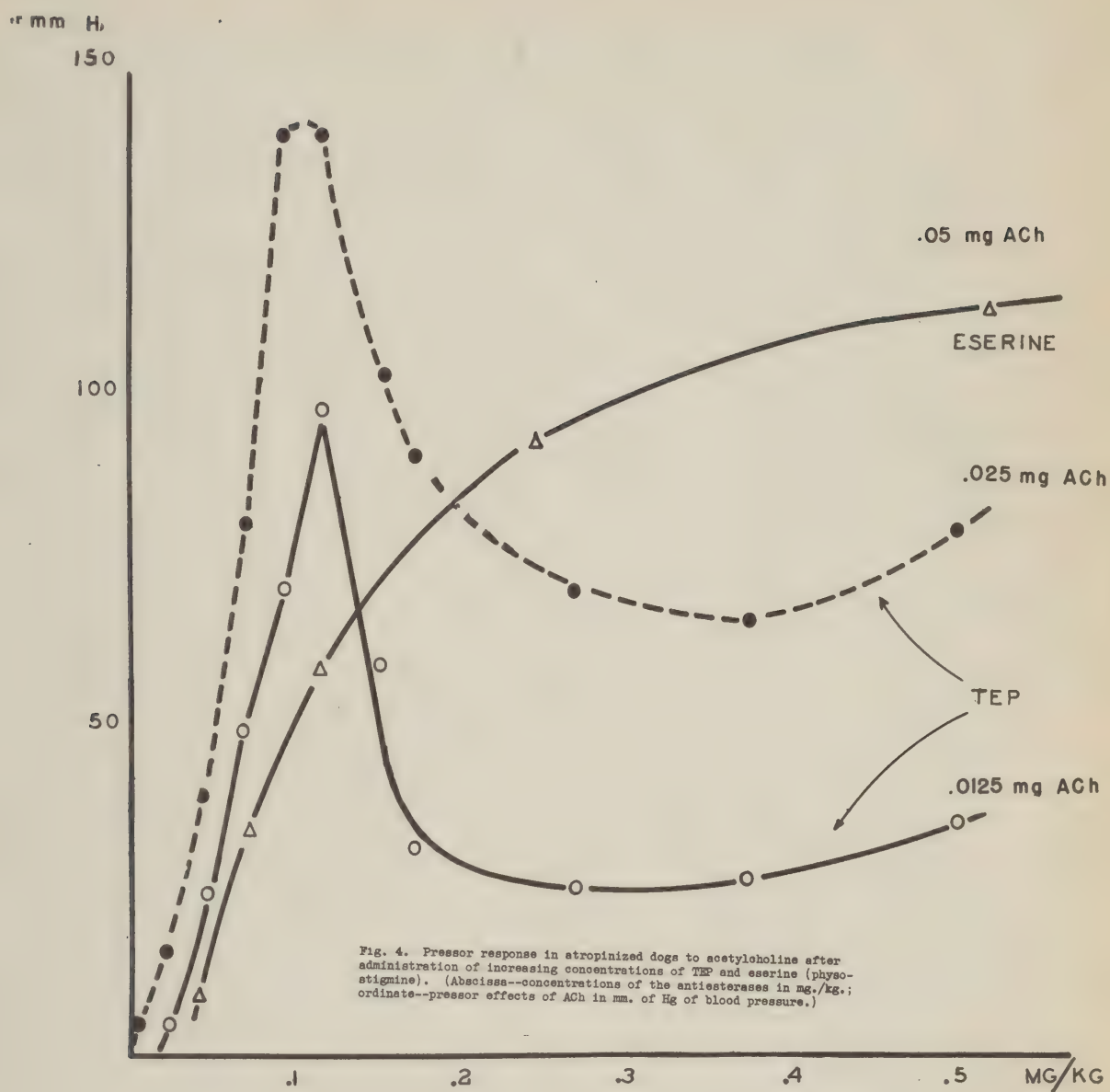


concentrations necessary for the second peak response. It is assumed accordingly that the 100% inactivation of the esterase occurs earlier and coincides with the first peak of our curves, and that the second peak is probably due to other phenomena, to be discussed later.

(2). The earlier parts of the DFP and TEP curves seem to show a first degree relationship, although the slopes of the two pertinent equations are widely different. The activities of HETP, physostigmine and neostigmine (fig. 2,5) on the other hand seem to follow exponential curves. Both DFP and TEP seem to have a wide range of concentrations over which the acetylcholine response is fairly constant; however, it has to be remembered that the constancy of the pressor effect elicited by acetylcholine coincides with the curve's asymptotic part of the DFP curve and with the second peak of the TEP curve which, in this case, can be called, more justifiably, a plateau. It remains for further investigation to show whether the differences described for the quantitative effects of different antiesterases correspond to their basically dissimilar mode of action.

One of the causes of such dissimilar effects which could be ascribed to the antiesterases of the group studied here may lie in the fact that while neostigmine and physostigmine resemble, structurally, choline esters and, therefore, acetylcholine, also, the other antiesterase agents are chemically different structures, being inorganic (unpublished) and organic phosphate esters. Indeed, it has been shown that neostigmine alone not only stimulates the striated muscle whose cholinesterase has been inactivated by DFP (1, 12) but also elicits a pressor effect in atropinized dogs, in concentrations from 0.1 mg./kg. upwards. This effect is not unlike the "nicotinic" action of acetylcholine in atropine-premedicated dogs. It can be shown, however, that this effect of prostigmine differs from that of acetylcholine in at least one important aspect. While the acetylcholine pressor effect can be potentiated by DFP, the neostigmine effect cannot be enhanced by this esterase inhibitor. This type of an experiment should be performed with a great deal of care, because the test doses of neostigmine may interfere with the physiological activity of DFP. Therefore, sufficient time must elapse between the injection of the test doses of acetylcholine and prostigmine until the acetylcholine effects (which were potentiated by the test doses of neostigmine) disappear due to the elimination of the antiesterase (usually six hours following the administration of not more than 0.4 mg./kg. of neostigmine). DFP can now be administered with safety (from 5 to 10 mg./kg.) and the marked pressor effect of acetylcholine obtained shows that DFP has taken effect. Then the test doses of neostigmine are injected repeatedly and the responses recorded. The pressor effects of the latter drug, in sharp contrast to those of acetylcholine, are not potentiated by DFP.

While DFP, and probably other phosphate esters suffer definite loss of activity (evidenced by the increase of LD<sub>50</sub> for DFP and HETP and the in vitro inhibition of their anticholinesterase effects) when





administered following the injection of moderate doses of physostigmine, the administration of physostigmine or neostigmine in sub-minimum or small doses enhanced the activity of DFP (2,13). During the course of these studies it has been shown that relatively small doses of physostigmine interfere with the acetylcholine-potentiating effect of DFP, while the administration of physostigmine or neostigmine following DFP causes a further potentiation of the acetylcholine pressor effects. This latter combined action of DFP and physostigmine appears to be potentiation rather than mere addition of effects.

A further proof of the different nature of the pressor effects of acetylcholine and neostigmine is furnished by experiments in which increasingly larger doses of neostigmine were administered. The magnitude of the pressor effect of neostigmine varies as the size of the dose. At a point when sufficient amounts of neostigmine have been accumulated to interfere with acetylcholine pressor effects, blood pressure elevations following the injection of individual doses of neostigmine were still undiminished.

Several experiments employing various cholinesterases were carried to a point where massive doses of these agents caused diminution or even annulment of the pressor effect of acetylcholine. It may be argued that not the peak or plateau, but the extinction of the curve represents 100% inactivation of the esterase, since at this point an excess of accumulated acetylcholine at the effectors may prevent further stimulation of the responsive structures by injected acetylcholine. The peak or plateau of the curve was, however, taken arbitrarily as the 100% inactivation point, since this corresponds most closely to results obtained by other methods, and since severe toxic effects at the later stages could not be ruled out. The animals during the declining responsiveness to acetylcholine can be kept alive only by continuous artificial respiration and the appearance of cardiac slowing indicates myocardial damage. With massive doses of antiesterases, the intense cardiac slowing may be enhanced by injection of acetylcholine and bronchial constriction appears to take place. The effect of such doses of the esterase inhibitors must be further investigated.

### Discussion

The study of the effectiveness and mechanism of action of various cholinesterases in intact animals should be particularly welcome at this time when differences in pharmacological response to various cholinesterase inhibitors by the in vitro and the in vivo techniques are emphasized (14). These investigators found marked differences in the anticholinesterase activity when the inhibitors were added to homogenized rat tissue in calcium-free Ringer-bicarbonate medium, and when they injected rats with anticholinesterases, and removed the tissues for cholinesterase measurements. They postulated that penetration is an important factor in the activity of the above agents and emphasized the necessity for in vivo testing. A further fact obtained by the in vitro method and reported by Miller and Ginsburg (15) requires elucidation. To test the assumption that anticholinesterases owed their



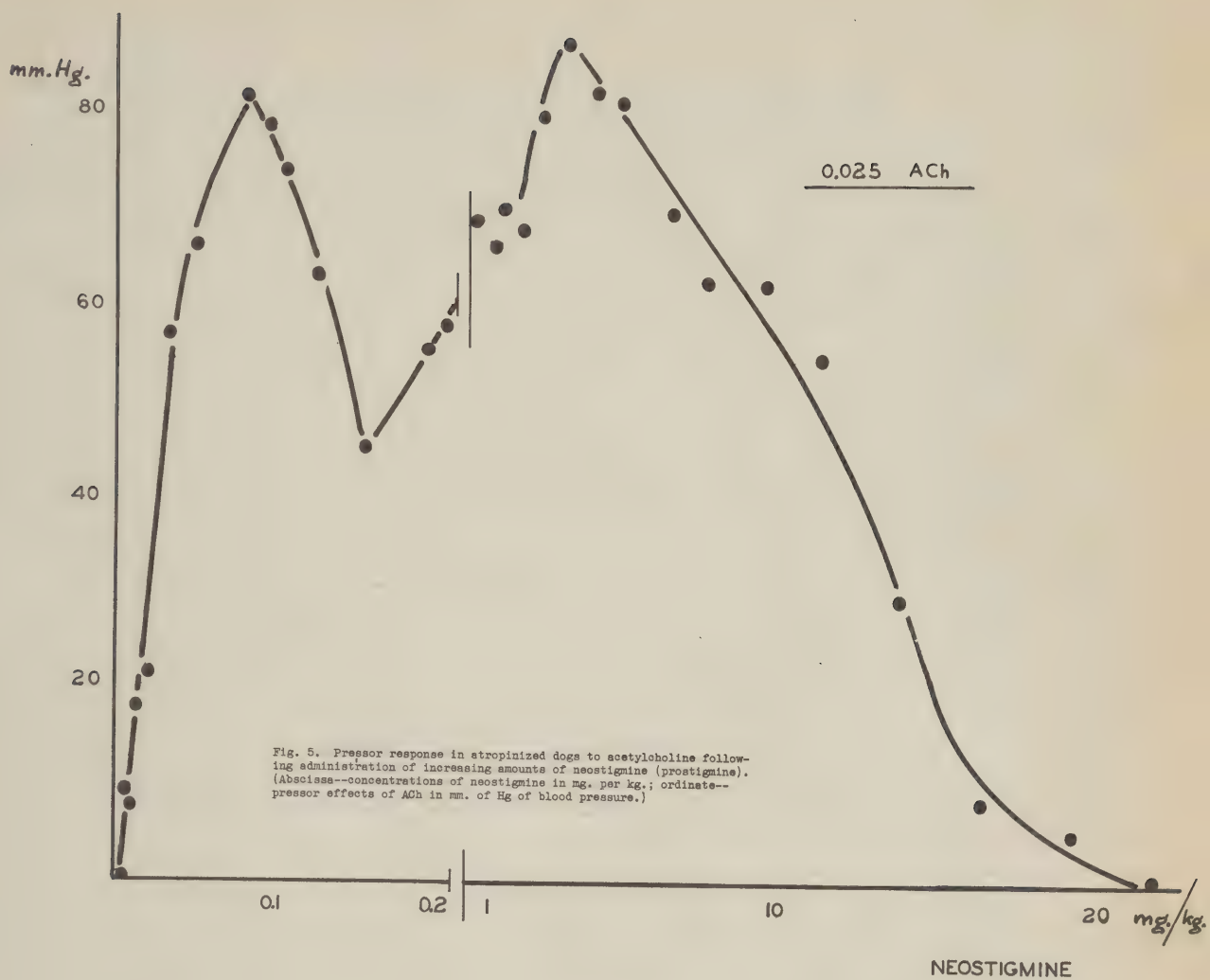


Fig. 5. Pressor response in atropinized dogs to acetylcholine following administration of increasing amounts of neostigmine (prostigmine). (Abscissa--concentrations of neostigmine in mg. per kg.; ordinate--pressor effects of ACh in mm. of Hg of blood pressure.)

activity to the accumulation of free acetylcholine (a strong probability when their effects on injected acetylcholine are recalled), they determined the free acetylcholine in the brain and in the submaxillary gland following injections of neostigmine and DFP. They found that while neostigmine produced an appreciable increase in free acetylcholine at the end-organs (salivary gland) but not in the brain, DFP caused a 3-fold increase of the brain acetylcholine, but only an insignificant rise of the neurohumor at the salivary effectors. It does not appear that the authors took advantage of the improved technique of acetylcholine estimations in isolated organs devised by Abdon & Hammarskjöld (16), but still their conclusion that the amount of acetylcholine liberated at the site of action of the antiesterase is not primarily responsible for the method of action of these drugs, is a disturbing feature and throws doubt on the hitherto generally accepted effect of esterase inhibitors.

The advantages of the method utilizing the responses of injected acetylcholine in the whole animal are many. The use of atropinized dogs for these experiments allows the employment of large doses of antiesterases, and it was thus discovered that the maximum effect of these drugs does not appear until large and, but for the presence of atropine, fatal doses were administered. This method also made possible the selection of one single pharmacological response to acetylcholine, and the quantitative measurement of the pressor effect in terms of mm. of Hg. Finally, this method is free from errors that may be encountered in extractions and chemical titrations, and it is simple and quick. Also it is possible to apply it to higher mammals, permitting predictions as to the therapeutic and toxic effects of these compounds in man.

Obviously, experiments in intact mammals cannot differentiate between individual cholinesterases. One cannot say what happens to the cholinesterase in the serum, in the glands, smooth muscles and the nerves, or in the brain. It is possible, however, to make use of the concept of essential cholinesterase, a term that should signify the total cholinesterase necessary for the proper functioning of any given cholinergic structure, in this case, the sympathetic ganglia. The data presented in this paper refer to the sum total of the esterase concerned with the elicitation of ganglionic effects.

In correlating the above experimental results with the mechanism of action of the esterase inhibitors, it appears that DFP acts only by irreversible inhibition. The larger its dose, the greater its effect, and relatively large amounts are necessary to produce both the initial and the maximal ganglionic effects. We found no evidence of ganglionic depression under the above experimental conditions except at concentrations approaching lethal. HETP is apparently another irreversible inhibitor, but while the restitution of the esterase following DFP takes about from 14 to 30 days (17), the recovery of the

original esterase level following HETP is accomplished from 7 to 10 days (18).<sup>3</sup> The latter, however, is far more potent than DFP, perhaps indicating better penetration. In any event, the experiments here reported show that inhibition of the esterase alone is sufficient to potentiate acetylcholine pressor responses. On the basis of its chemical structure, TEP should behave similarly to DFP and HETP, but it differs from them in two important respects: (a) it is the most potent of the anticholinesterases, and the steepness of its activity curve resembles that of the neostigmine curve, (b) like physostigmine and neostigmine it shows a double-peaked curve when its concentration is plotted against the pressor effects of acetylcholine. It is tentatively suggested, therefore, that TEP owes its effects primarily to anticholinesterase activity, but other actions may also be involved.

Physostigmine and neostigmine are very similar in that they are potent and rapidly acting compounds and that the 100% essential esterase inactivation occurs early, followed either by a plateau or by a temporary decrease of the acetylcholine pressor response with increasing doses of the esterase inhibitors. Then the acetylcholine pressor effects return to normal, or even supranormal levels, but this phenomenon is observed at esterase levels which correspond neither to the in vitro 100% inactivation point nor to the dosage levels of these esterase inhibitors at which intense pharmacological effects, in addition to blood pressure elevations, are obtained (muscle twitches, respiratory stimulation, etc.). This suggests that the first peak is due to cholinesterase inactivation, and the second to what may be called an independent potentiation of acetylcholine by the antiesterases, probably due to a toxic effect on cellular surfaces and/or to permeability changes. Permeability changes by esterase inhibitors have been reported by various authors. A further indication of this contention is the fact of potentiation of DFP effects by physostigmine or prostigmine, adequately demonstrated by Koppanyi, et al. (2), and Miquel (1).

The irreversibility of action of the phosphate esters should be further studied on atropinized dogs using acetylcholine pressor effects as criteria. Such experiments have already been performed in this laboratory with reference to physostigmine and neostigmine. It is probably safe to state, however, that every esterase inhibitor should be investigated for its ganglionic effects to determine what part of the action is due to esterase inhibition alone, and what part to other factors.

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<sup>3</sup>It should be pointed out, however, that Chase, Schmidt and Bhattacharya (19) showed in the intact rabbit that the anticurare action of DFP is slightly diminished after one hour, while at the end of the first day after injection, the antidotal effect of DFP suffered a 50% and on the fifth day an 80% loss. On the twelfth day the effectiveness of DFP had practically disappeared. This observation is an additional compelling reason for the introduction of the concept of essential or specific cholinesterase.



The "independent" direct effects of neostigmine, such as muscular contraction or rise in blood pressure, are not potentiated by DFP so that they cannot be caused by changes in esterase activity. The effects of physostigmine and neostigmine on acetylcholine potentiation are partly due to antiesterase action, but may also occur independently since in a large series of experiments minimal or sub-minimal doses of physostigmine enhanced beyond expectations the acetylcholine-potentiating actions of DFP. Heymans ('46) apparently concurs, but unfortunately he worked with muscarinic responses and the concentrations of DFP employed by him were so small that they did not produce detectable cholinergic phenomena. His experiments may only prove simple addition of the effects of DFP and physostigmine.

### Summary

1. Newer phosphate esters (DFP, HETP, and TEP) with marked anti-esterase activity potentiate acetylcholine pressor responses in atropinized animals.

2. Physostigmine appears to inhibit the DFP effect on acetylcholine pressor responses, while physostigmine and neostigmine cause further potentiation of such pressor effects when administered after instead of before DFP medication.

3. The comparison of the curves relating the concentration of various antiesterases to the pressor effects of standard doses of acetylcholine reveals marked differences in potency. Their slopes differ. The curves for physostigmine, neostigmine and HETP are exponential in character, while the relationship for DFP and TEP is first linear; then, over a wide range of concentrations, there is no change in the magnitude of acetylcholine responses. The range of concentrations of DFP over which maximum acetylcholine pressor effects appear is identical with the asymptotic part of the curve, while with TEP this part of the curve is a plateau.

4. Physostigmine, neostigmine and TEP produce a temporary depression of ganglionic responsiveness to both acetylcholine and nicotine which is probably due to ganglionic depression. This effect disappears as soon as the concentration of the antiesterases in the body increases.

5. The mechanism of action of antiesterases, particularly those of the physostigmine type is related, in addition to esterase inhibition, to physiochemical changes in the membranes concerned.

6. The advantages of the quantitative study of nicotinic responses in the intact atropinized dog is stressed, and the concept of essential or specific cholinesterase is introduced.

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## THE ENZYMATIC CONVERSION OF CYANIDE TO THIOCYANATE (I)

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The minute amounts of thiocyanate normally present in urine, blood and saliva have stimulated the interest of many workers as to the origin of the cyanide and the pattern of conversion of cyanide to thiocyanate. The problem has toxicologic as well as a physiologic importance because of the great toxicity of cyanide. The detoxication of cyanide in the animal body was first demonstrated by S. Lang (1,2) who was able to show that after the injection of cyanide or of aliphatic nitriles in the rabbit an increased amount of thiocyanate was excreted in the urine. Similar findings were reported by Heymanns and Mesoin (3). The formation of minute amounts of cyanide from products of protein metabolism and from the nitriles ordinarily present in foods and the conversion of that cyanide to thiocyanate was believed by these authors to account for the thiocyanate normally excreted from the body. The in vitro studies of this mechanism were initiated by Pascheles (4) who showed that liver and muscle tissue from the dog were able to produce thiocyanate after digestion with sodium cyanide, liver being more active in this respect than muscle. Kahn (5) concluded from this work and from a series of liver perfusion experiments in which the amount of thiocyanate produced increased with the number of perfusion trips, that the liver was an active factor in the production of thiocyanate.

It was not until 1933, however, that Konrad Lang (6,7) carried out his experiments on the in vitro production of thiocyanate from cyanide in the presence of sulfur. He postulated that an enzyme was responsible for the conversion of cyanide to thiocyanate, and described it as heat-labile rapidly-acting with pH and substrate concentration optima. The enzyme, which he termed "rhodanese", was widely distributed in animal tissues and present in large amounts in the liver. Lang, therefore, suggested that the formation of thiocyanate was the principal route of detoxication of cyanide in the body and that the liver was the chief site of this detoxication. Cosby and Sumner (8) purified this enzyme to some extent and made further studies of its properties. This paper deals largely with the distribution of the enzyme in various organs and tissues. A description of its properties will appear elsewhere.

## Experimental

Tissues obtained from 7 dogs, 5 monkeys, 9 rats, and 9 rabbits were assayed for their enzyme content in terms of ability of 1 gm. of tissue to produce thiocyanate from cyanide. The most extensive work was done on the dog in which the suprarenals (cortex and medulla separately) liver, kidney, heart, lungs, skeletal muscle, pancreas, spleen, cervical lymph nodes, testes, ovaries, salivary glands, intestine (duodenum, jejunum), spinal cord (cervical, lumbar, sacral regions separately), brain, (cortex, caudate nucleus, a mid-brain section (hypothalamus, thalamus and pons), cerebellum and medulla separately), the optic nerve, the epididymis, thyroid, eye, anterior pituitary, and blood components were tested.

The animals were rendered unconscious either by injection of pentobarbital in the case of dogs or by a blow on the head in the case of smaller animals. Monkey tissues were obtained from animals which had received either curare or pentobarbital or both. Tissues were removed from the body as rapidly as possible and homogenates prepared in 10 volumes of distilled water. Tissues weighing 10 grams or more were homogenized in a Waring Blender and filtered through two thicknesses of cheesecloth; those tissues weighing less than 10 grams were homogenized by hand.<sup>1</sup> The homogenates were stored in the refrigerator at 5°C.

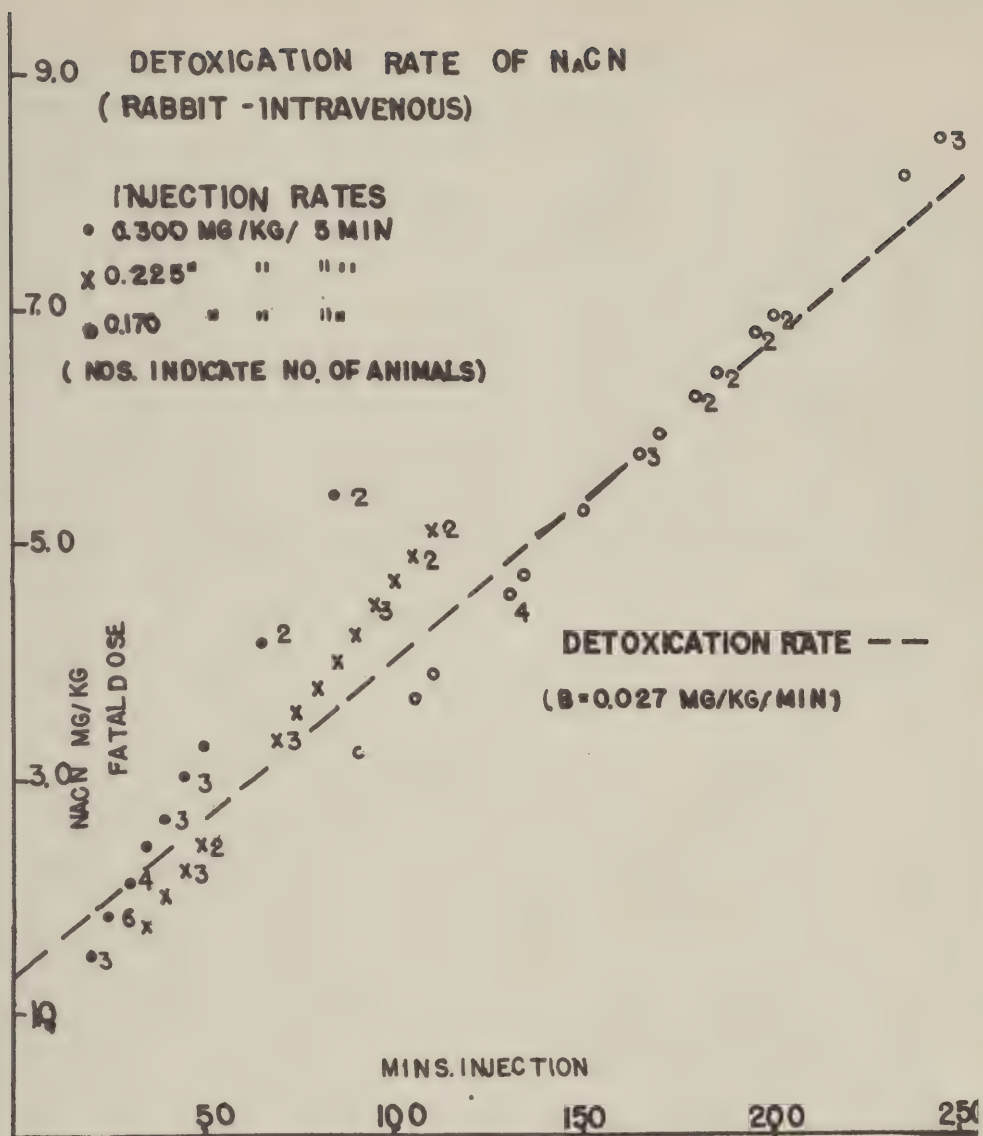
Preliminary observations indicated that a system containing phosphate buffer pH 7.4, 0.3 cc. tissue homogenate, 0.42 M sodium thiosulfate, 0.14 M KCN in a total volume of 9.2 cc. added in the order named and shaken for 15 minutes at 37.5°C gave conditions reasonably near the optimum for all tissues. The enzymatic activity was stopped by adding 10 cc. of a ferric nitrate-nitric acid solution exactly 15 minutes after the addition of the KCN. The reaction mixture was then diluted to 25 cc. with distilled water, shaken, centrifuged and allowed to stand 15 minutes to permit the violet color formed by the excess thiosulfate to fade. Thiocyanate was determined spectrophotometrically, a standard curve being prepared by adding known amounts of thiocyanate to the tissue system in the absence of cyanide.

Since the color of the iron-thiocyanate complex was too intense at concentrations greater than a few micrograms, it was necessary to dilute tissue homogenates which formed more than this amount of thiocyanate under the standard conditions shown above. The validity of such dilution is shown in figure 1.

Fourteen different sulfur-containing compounds of physiologic and pharmacologic interest were tested for their ability to replace thiosulfate in the standard system. The compounds were added in such amounts that they could furnish the same quantity of sulfur as did the thiosulfate. Slices, minces, and homogenates prepared from

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<sup>1</sup> Ace glass tissue homogenizer





representative samples of the dog and rabbit liver and of rabbit cerebral hemispheres were compared in respect to ability to form thiocyanate in the standard system.

## Results

The dogs studied showed a wide variation in the enzyme content of their tissues (table 1). However, in all dogs irrespective of the absolute value obtained, certain tissues always ranked highest and others always contained minimal amounts of the enzyme. The suprarenal gland had the highest enzyme content, the activity being almost entirely concentrated in the cortex. The dog apparently is unique, as the suprarenals from other species showed no great concentration of the enzyme as compared with other organs. Of the latter, liver was the highest although its value was often as low as a fifth of that of the suprarenals. The various parts of the brain and spinal cord, the kidney and testes had relatively large amounts; other tissues, such as heart, intestine, spleen, lung, muscle, and salivary gland, had smaller quantities, while that present in red blood cells and plasma was barely measurable. The two female dogs had the highest concentration of enzyme in the liver, in one the liver value was even greater than the suprarenal. Unfortunately, not enough females were available to establish a sex difference.

The monkeys studied contained more enzyme in the liver than in any other organ, the kidney being the next highest. The content of these organs was much higher than in the dog. Heart, and muscle also, contained higher concentrations than the dog tissue. The other organs, and the parts of the central nervous system, however, ranked with the same tissues from the dog.

Rabbit homogenates tended to have a little higher enzyme activity than those of monkey organs. The difference, however, was again not marked in the parts of the central nervous system. The same may be said for the rat -- liver and kidney contained higher concentrations of enzyme than were present in the other species but the parts of the central nervous system were about the same as in the dog.

In general, it can be said that while the enzymatic activity of liver, kidney, muscle and suprarenals vary markedly from specie to specie, the activity of the parts of the brain does not. The species grouped according to increasing activity of their liver and kidney homogenates are dog, monkey, rabbit, and rat.

Table 2 compares the enzymatic activity of slices, mince and homogenates prepared from the same tissue. The minced tissue converted approximately twice as much cyanide as did the sliced while the homogenate made approximately 5 to 17 and 3 to 8 times as much thiocyanate as did the sliced and minced tissues, respectively.

The ability of other sulfur-containing compounds to replace thiosulfate in the system is negligible. The best replacement was obtained





with sulfide, thiourea, and -naphthylthiourea, but no conversion occurred with the sulfur-containing amino acids (table 3).

Table 2. MG. -CN Converted to -CNS by 100 MG. Tissue

	<u>Rabbit Cortex</u>	<u>Rabbit Liver</u>	<u>Dog Liver</u>
Homogenate	109.6	514.0	3.70
Slice	7.1	30.5	7.3
Mince	12.8	71.7	11.0

### Discussion

The enzyme responsible for the conversion of cyanide to thiocyanate is widely distributed in the animal body and in relatively large amounts. Mendel et al. (9) report a different distribution pattern than we do. This discrepancy may be due to the fact that they did not use the same amounts of cyanide and thiosulfate that we did and that they studied only tissues from the rat, whereas, most of our data is based on the dog. On the basis of their data they suggest that this enzyme is concentrated in those tissues whose metabolism would be markedly inhibited by small amounts of cyanide, for the purpose of removing any traces of cyanide formed in metabolism. Our distribution data on tissues from dogs and other species do not permit us to concur with their opinion and we feel that an enzyme so widely distributed in such large quantities has some metabolic function other than the detoxication of cyanide.

It is difficult to make a comparison between our data and Lang's (6) because he used an acetone powder, the preparation of which may have resulted in variable losses of activity and because he expressed his data in arbitrary units based on the activity of the dry powder. But, with one or two exceptions, our data on the distribution of activity in tissues of the dog seem to agree well with his. The data suggest that even though the liver undoubtedly plays a major role in removing cyanide, detoxication probably occurs in all parts of the body. The high activity of various parts of the brain taken together with the very large blood flow through that organ, and the large mass of muscle even though with a relatively low activity, should make brain and muscle important sites of detoxication.

In attempting to relate in vitro work to the intact animal who has been poisoned with cyanide, several questions present themselves. An in vitro system such as described here completely avoids the problem of cellular permeability. Cyanide is known to enter the cell readily and probably combines at once with the cytochrome oxidase present (10). Thiosulfate on the other hand probably penetrates more slowly, if at all, since Gilman et al. (11), have shown that 70% to 80% of ingested thiosulphate is excreted unchanged. The data presented



on the different activities of slices, mince, and homogenate indicate that the factor of permeability may play an important part in determining how much cyanide can be detoxified by an animal, irrespective of the amount of enzyme present.

Table 3. Replacement of Sodium Thiosulfate by other Sulfur Containing Compounds (Sodium Thiosulfate = 100% Activity).

<u>Compound</u>	<u>% Activity of Standard System</u>
Sodium Thiosulfate	100%
Sodium Sulfide	4
Sodium Tetrathionate	Spontaneous Conversion
Thiourea	4.5
- Naphthylthiourea	4.6
Thiouracil	1
Dithiobiuret*	1
Methionine	1
Cystine	1
Cysteine	1
Thiodiglycol	0
Diphenylsulfide +	0
Diphenyldisulfide +	0

\*Supplied through courtesy of American Cyanamide Company

+Supplied through courtesy of General Chemical Company

Calculating from the in vitro results, the amounts of cyanide which the tissues of the dog can theoretically convert to thiocyanate in a fifteen minute period are enormous. The whole liver of one dog studied could have detoxified 4,015 gms. of cyanide and the total skeletal muscle of the same dog, 1,763 gm.

These amazingly large amounts of cyanide with which a tissue can deal in vitro as compared with the relatively small size of a fatal dose suggest that availability of sulfur not quantity of enzyme present is the limiting factor in the detoxification. This concept is strengthened by the work of Chen, Rose and Clowes among many others

showing that injection of thiosulfate is capable of increasing the LD<sub>50</sub> as much as 3 to 4 times (12). In vitro at least the conversion of cyanide to thiocyanate does not proceed efficiently unless a thiosulfate concentration of at least three times the molar concentration of cyanide is present. That such a concentration of thiosulfate exists normally in the cell is doubtful. There is no doubt, however, that sub-lethal doses of in vivo administered cyanide is excreted almost completely in the urine as thiocyanate (13). Our data do not suggest that any of the tested compounds other than thiosulfate is effective as a sulfur donor, thus leaving the question of the source of sulfur in the cells for formation of thiocyanate still unanswered. Tissue homogenates in our procedure did not contain or could not make sufficient thiosulfate even over a period of eighteen hours to convert a measurable amount of cyanide to thiocyanate. It is probable, however, that our treatment of the homogenate resulted in loss of the enzyme system, which Smythe (14), Fromageot (15), and Garabedian (16) have suggested converts the sulfur of amino acids to sulfide and then to thiosulfate since that system is known to be unstable. Such a formation of thiosulfate, however, would probably proceed too slowly and be too limited by the quantity of sulfur-containing amino acids present to permit any but slow detoxication.

Although the content of enzyme of the various species varies greatly in liver, kidney and suprarenal glands, that of the central nervous system does not. These data may account for the fact that the LD<sub>50</sub> for intravenous injected sodium cyanide is approximately the same for all these species. On the other hand, the difference in enzyme activity of the livers from two of the species tested appears to have a relation to the rate at which cyanide is detoxified when given in sub-lethal doses. Mukerji and Smith (17) reported that rabbits were able to detoxify cyanide quite rapidly - nearly all the cyanide being recovered as thiocyanate in the urine within 24 to 48 hours. In the dog, however, less than 25% of the injected cyanide was recovered within a period of 7 days.

#### Summary

1. The distribution of the enzyme capable of converting cyanide to thiocyanate in homogenates prepared from tissues of the dog, rhesus monkey, rabbit and rat was studied.

2. No marked species difference was observed in the activity of homogenates prepared from parts of the central nervous system. Other tissue homogenates, however, showed a wide variation; the activity increasing in the following order: dog, rhesus monkey, rabbit and rat.

3. The thiosulfate ion was the only sulfur containing compound found capable of efficiently providing sulfur in the in vitro system.

4. Data is presented to show the intracellular character of the enzyme responsible for thiocyanate production.

5. The significance of the enzyme system in connection with the in vivo detoxication of cyanide is discussed.

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## THE SUSCEPTIBILITY OF RABBITS TO CYANIDE

(AS AN EXAMPLE OF DETOXICATION STUDIES)

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During the past several years, personnel of the Toxicology Section have studied detoxication of various compounds. The increase of dose necessary to produce a certain mortality as the rate of dose injection is decreased, has been used as a gross measure of detoxication. The word "detoxication" is used in a broad sense to indicate disposal of the toxic compound.

The intravenous injection of solutions of NaCN into rabbits<sup>1</sup> provides a general example of the methods used, the reproducibility of results and the variation between animals.

When the duration of the period of injection of a fatal dose is plotted against the fatal dose injected the following is noticed:

1. A line drawn through the points where a certain percentage of animals have died at each injection rate has an intercept at zero time corresponding with the acutely injected dose producing the same mortality. This intercept for 50% mortality is about 1.4 mg. NaCN/kg. in rabbits.

2. This line is a straight line in the present case and, therefore, within the limits of the lowest rate of injection used (170  $\mu$  NaCN/kg./5 min.) the detoxication rate is a constant one of 27  $\mu$  NaCN/kg./min. (If the curve were a hyperbola, a detoxication rate decreasing with time would be indicated).

3. A line drawn through the points for any percentage mortality is parallel to that for any other percentage mortality, thereby indicating that the rate of detoxication of NaCN must be nearly the same in all rabbits.

4. Since the detoxication rates appear to be nearly the same in all rabbits and since the intercepts for any certain percent mortality correspond with those for the same percent mortality after an acute dose of NaCN, then the following is true: when, from the total

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<sup>1</sup>H. J. Pratt, R. G. Horton, J. D. Zierler, Medical Division Report #26, 10 March 1945.

dose for each rabbit,  $27 \text{ y NaCN/kg./min.}$  is subtracted, the remainder,  $\underline{k}$ , constitutes the dose actually killing that rabbit and when each and every  $\underline{k}$  at any one rate of injection is plotted as the log of the dose against the cumulative percent mortality expressed as probits, a dosage-mortality curve is formed which has characteristics similar to the acute dosage-mortality curve both as to its errors and slope.

5. The above method is merely another expression of the familiar  $(Ct) - (dt) = \underline{k}$  where

$C = \text{dose/unit time}$

$t = \text{total units of time during which fatal dose was administered.}$

$d = \text{amount detoxified/unit time.}$

6. Whether injections of NaCN were given constantly by a machine, or intermittently at 5 minute periods by hand injection, similar results were obtained.

Through such studies accurate estimates may be made of the rate of detoxication, the constancy of the detoxication rate and comparative rates in various species. Such gross estimates form the starting point for more detailed studies of the disposal of a toxic material by various routes such as the respiratory tract, kidney, conversion to non-toxic forms, etc. Comparisons may be made with the detoxication rate as determined from in vitro studies. For instance, the in vitro work of J. P. Saunders and W. A. Himwich in our Section with tissue homogenates indicates that the potential detoxication (by conversion to -CNS~~y~~) rate of NaCN in rabbits is many times greater than  $27 \text{ y NaCN/kg./min.}$  However, due presumably to the slow movement of metabolites, such as the thiosulfate ion, through the cell walls, the full potentialities of detoxication of NaCN are never reached.

Although large numbers of rabbits were used so that the present study enables one to make statistical comparisons with little error in order to substantiate the principles discussed, once one has accepted these principles, merely a few animals used over a wider dose rate range would permit the establishing of a satisfactory estimate of the detoxication rate of any compound.



# DISPARITY BETWEEN THE CLEARANCES OF CREATININE AND INULIN BY DAMAGED KIDNEYS

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It was found in both rabbits and dogs exposed to inhalation of uranyl compounds that the clearance of diodrast could become smaller than that of inulin. It has been shown by Bobey et al (Am. J. Physiol. 139: 155, 1943) that after intravenous injection of uranyl acetate the renal blood flow falls only slightly. The fall of the diodrast clearance below that of inulin in our experiments means probably that tubular secretion of diodrast was abolished completely for a time and that the tubular epithelium became increased permeable to back diffusion of dissolved substances and water from the tubular lumen into the peritubular capillaries. Diodrast (molecular weight 510) would be expected to show such back diffusion more prominently than inulin (mol. wt. about 4500).

To test the hypothesis of altered physical properties of the tubular epithelial lining, 6 rabbits were injected intravenously with uranyl acetate and their simultaneous renal clearances of creatinine (mol. wt. 113) and inulin were determined at intervals. It was found that the creatinine clearance fell from its normal value more rapidly than that of inulin although both trended toward the same final value, so that the ratio of the inulin clearance to that of creatinine went through a maximum. This indicates that slight damage of the tubular epithelium by uranium increased its permeability to creatinine without altering that to inulin. As the damage increased, the tubular epithelium became permeable to both substances and eventually both inulin and creatinine were back diffusing freely.

These findings reinforce the idea that clearances by damaged kidneys can not be interpreted by the same scheme which applies to normal ones.

This work was done at the University of Rochester under the Manhattan Project of the Corps of Engineers. The analytical determinations were carried out by Dr. Edna Main.



OBSERVATIONS ON BONE MARROW REGENERATION AND  
BLOOD IN CHRONIC PHENYLHYDRAZINE ANEMIA IN SWINE

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It is well known that an increase in the reticulocyte percentage will affect the mean measurements of corpuscular size. This is usually a temporary effect following a sudden loss of blood, a hemolytic crisis, or any reaction which acutely stimulates the hematopoietic system. This study was carried out in an attempt to correlate the reticulocyte percentage and the mean corpuscular volume in swine under controlled conditions.

Six adult swine were hematologically studied and periodic bone marrow evaluations were made during a period from 15 to 50 days while they received large amounts of phenylhydrazine hydrochloride orally and intravenously. One animal died on the 15th day of treatment with the drug and exhibited a course which closely resembled that of benzol poisoning. Rapid and progressive granulocytopenia, anemia, and extreme universal hypoplasia of the bone marrow was observed in this animal. The 5 remaining swine responded to the drug in the usual manner with progressive anemia, reticulocytosis, and erythrocytic hyperplasia of the bone marrow. The other commonly associated hematological findings were observed.

A straight line correlation between the mean corpuscular volume of the red cells and the percentage of reticulocytes was found. With each increase of 10% in the reticulocyte count, the mean corpuscular volume increased approximately 6.5 cubic microns.





# PHYSIOLOGICAL ACTION OF PREFERENTIALLY INACTIVATED INSULIN

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Since the discovery of insulin and particularly since the demonstration by Abel (1) that it could be prepared in pure crystalline form, the effect of various chemical and physical alterations of the insulin molecule on its endocrine activity has been a subject of much interest. Treatment with alkali, acid, acid alcohol, and iodine; acetylation, formation of azo derivatives, irradiation and many other procedures have been shown to reduce or completely destroy its hypoglycemic action. Such experiments were designed to find out what specific group or groups were essential for insulin's activity. The theoretical and practical value of knowing this is obvious. The multiplicity of chemical modifications which will cause loss of activity, however, now almost forces us to the conclusion that no single group or structural unit is important by itself, but that the detailed structure and spatial arrangement of the entire insulin molecule is the basis of its activity.

Beginning in 1935, Bürger and his coworkers (2,3) reported the use of chemical methods of inactivating insulin for a slightly different purpose. It is well known that when insulin is given intravenously to animals, the blood glucose is increased for a few minutes before it starts to fall. Since Bürger did not find this initial hyperglycemia to be prominent when crystalline insulin was used, he concluded that it was the effect of a separate substance. He inactivated ordinary commercial insulin and showed that although the hypoglycemic action of the insulin had been destroyed, his hyperglycemic factor was unaffected and its action on the blood sugar was actually potentiated by the removal of insulin. Although most of his work was done by boiling insulin with dilute sodium carbonate, he showed that reduction by cysteine, a procedure described by Du Vigneaud (5) had the same effect. The substance he had demonstrated in this manner he named "Glukagon" and believed it was a normal endocrine product of the pancreas. He even suggested that it might be operative in certain diseases and that diabetes represented not a deficiency of insulin alone, but rather a sort of imbalance between insulin and glukagon. It is to be emphasized, however, that Bürger's argument for the separate existence of a hyperglycemic hormone of pancreatic origin was by no means complete. He never was able actually to isolate his factor, but could only demonstrate that its effect was present in mixtures which included a certain amount

of inactivated insulin. The possibility still exists that the hyperglycemic effect was due to some moiety of the insulin molecule itself or that it was in part the result of the chemical modification that had been effected.

The experiments which follow were designed in an attempt to throw some light on this question. The method of inactivation used was reduction with cysteine. This procedure, described by Du Vigneaud and extensively studied by Wintersteiner (8) is better understood from the chemical viewpoint than any other. The only chemical change is believed to be the reduction of the disulphide linkages of which there are probably 18 in the insulin molecule. We wished first to establish the effect Burger had described with commercial insulin available now in this country and secondly to determine whether any such effect could be elicited from pure crystalline insulin treated in the same way. In an attempt to achieve some insight into the mechanism of the hyperglycemic action, the material was tested on pancreatectomized as well as normal dogs and in the former, blood ketone levels were determined simultaneously with the blood sugars. In order to decide whether the hyperglycemic had true "anti-insulin" activity, mouse protection tests were also done.

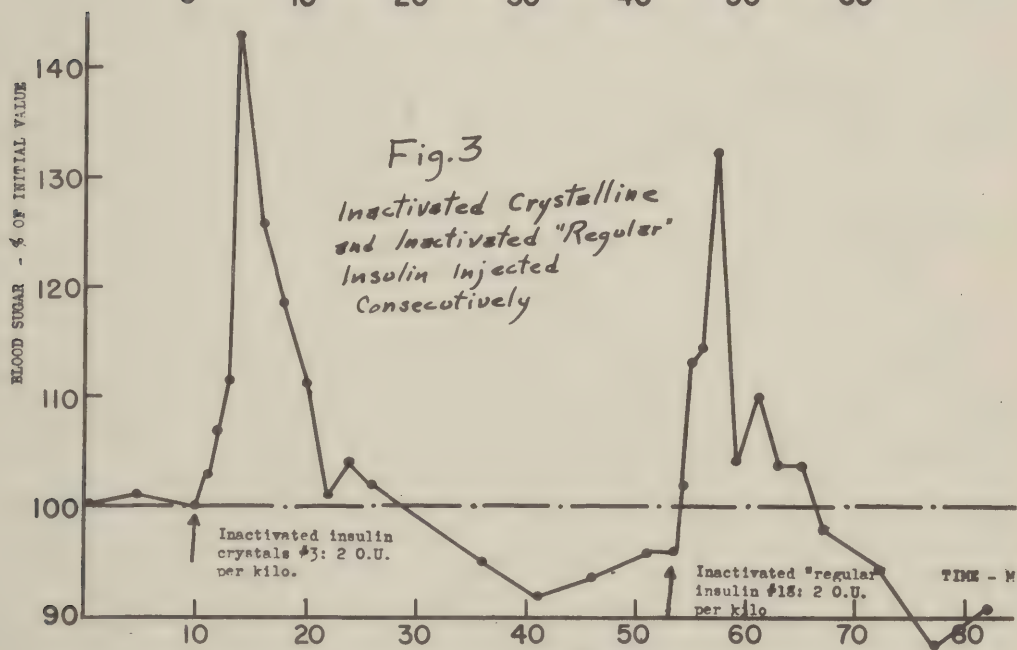
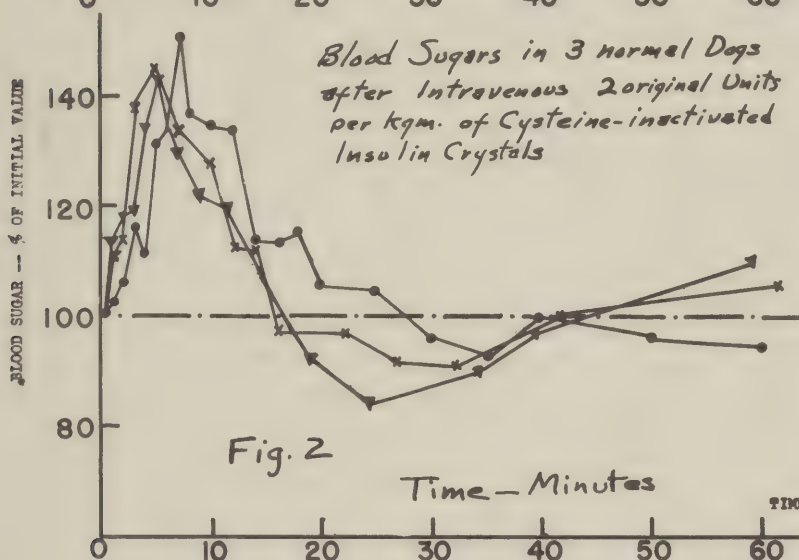
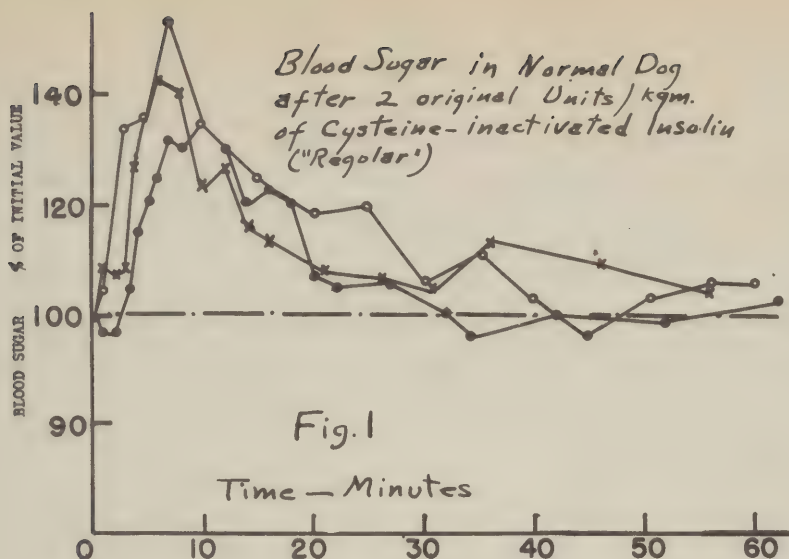
### Methods

For "Regular" insulin, Eli Lilly U-40 was used. Solutions of crystalline insulin were prepared from dry Zinc-insulin crystals kindly donated by the Eli Lilly Company. The inactivation procedure was as follows: 2 mgm. of cysteine for each milligram of insulin was added to the solution and the pH adjusted to 8.5 with 0.1 N sodium hydroxide. The mixture was allowed to stand under nitrogen at room temperature for 24 hours, at the end of which time the pH was adjusted to 4.0 and the protein precipitated with half-saturated ammonium sulfate. It was then centrifuged and the supernatant discarded, washed with phosphate buffer at pH 5.8, centrifuged again and dissolved in water with the addition of small amounts of 0.1 N sodium hydroxide. It was then dialyzed against water and finally dissolved by dialysis in whatever solution it was to be used for administration.

Assays for residual activity were carried out by the statistical mouse-convulsion method (7). In 16 samples prepared in the above manner, the residual activity ranged from 0.1 to 1%. Blood ketone determinations were carried out by a modification of the Behre-Benedict salicylaldehyde method which we have described elsewhere (4,9), and blood sugars were determined on venous samples by the Folin micro method (6).

In all cases, trained dogs were used which tolerated repeated venous punctures without excitement. After 3 or 4 "base line" samples had been collected, the material to be tested was injected intravenously. In general, a sample was drawn every minute for the first 10, every 2 minutes for the next 10 and every 5 or 10 minutes from then on.





## Results

Hyperglycemic effects. Figures 1 and 2 show characteristic blood sugar curves following the injection of inactivated "regular" insulin in the first instance and crystalline in the second. The points are plotted as percent of the base-line value rather than in mgm. % in order that they may be more easily compared. In 10 such runs with reduced amorphous insulin, the mean maximum was 144%. In ten similar runs with crystalline material treated identically, the mean maximum was 137%. It cannot be said that this difference is significant, since by Student's "T" test, there is a probability of greater than 0.1 that the two means belong to the same distribution. Figures 3 and 4 illustrate experiments in which the two types of preparations were injected successively, the second being given as soon as the blood sugar had time to reach its approximate base-line level.

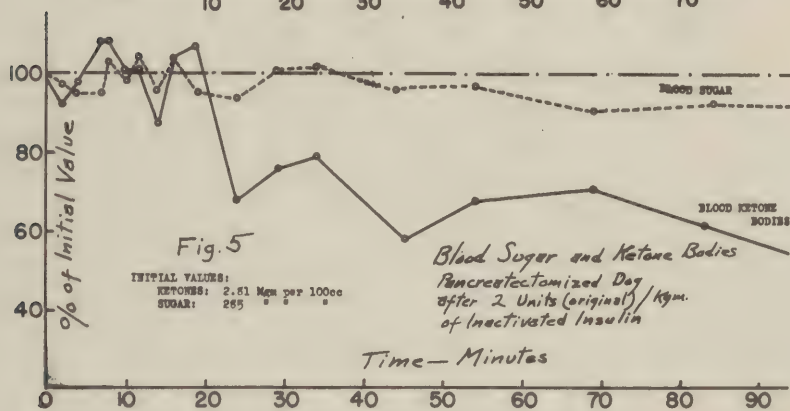
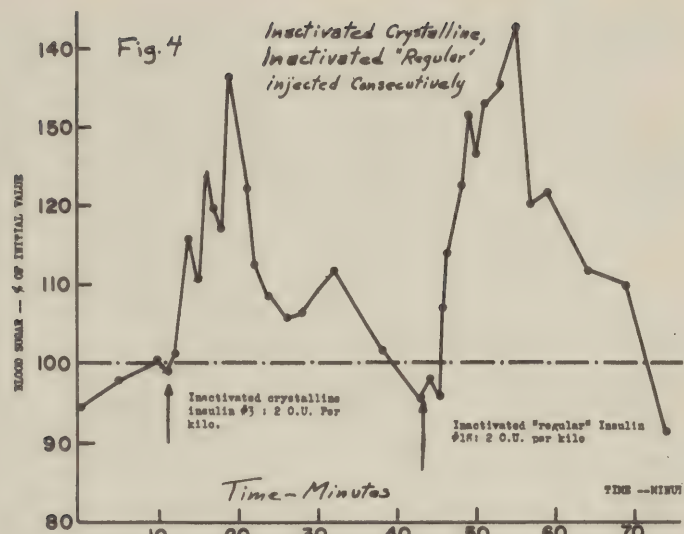
Effect on pancreatectomized dogs. Figures 5 and 6 illustrate the influence of cysteine-reduced insulin on the blood sugar and acetone bodies of pancreatectomized dogs which had not received insulin for periods of 18 to 48 hours. It is apparent that the hyperglycemic effect is slight and of short duration. In 7 such experiments, the maximum increase ranged from 1 to 20%, the effect in general becoming smaller as the insulin deprivation period was extended.

The ketone levels shown on these are perhaps not of much significance. The over-all drop in ketone concentration in one or two hours cannot be considered to be the result of the injected material since controls show that in pancreatectomized dogs which have been fasted for 18 hours, the ketone levels may be falling very rapidly. It does seem to be important that an increase in ketone concentration tends to occur during the short period in which the blood sugar rises.

Effect on susceptibility of mice to insulin-induced convulsions. In figure 7 the dotted line shows the relationship between insulin dosage and percentage convulsions in groups of fasted white mice kept at 37° C. The other four curves describe this relationship for mice which have been "protected" by the simultaneous administration of 40, 60, 120, and 160 milliunits of reduced insulin. Each point represents a group of 24 or more mice. It is apparent that there is no protective effect. The convulsion rate is actually slightly higher in most of the treated groups.

## Discussion

In view of the similarity of the results obtained by inactivating "regular" and crystalline insulin, it is apparent that the hyperglycemic principle which Bürger described is not, as he supposed, merely a contaminant of amorphous insulin which is removed by crystallization. It must be either a part of the insulin molecule itself or a substance so closely related to insulin that it is not removed by ordinary methods of crystallization. It is also not clear to what extent hyperglycemic activity is induced by the process of inactivation itself.





In considering the mechanism of action of the hyperglycemic principle, the question arises as to whether it operates by interfering with the action of insulin. It is an attractive hypothesis, for example, that a substance very closely related to insulin could take its place in some chemical situation and thereby block insulin without being able to carry out its metabolic function. If this were the case, however, there should be some dose of inactivated material which would protect against the convulsant effect of insulin. This we have been unable to demonstrate.

We are therefore left with the theory that this principle has its own independent influence on the regulation of the blood sugar. Bürger suggested that it operated by merely liberating hepatic glycogen stores. This may well be the case. The observations that the responses are smaller in untreated diabetes and that increases in blood ketone levels parallel the increases in blood sugar conform well to this hypothesis.

### Summary

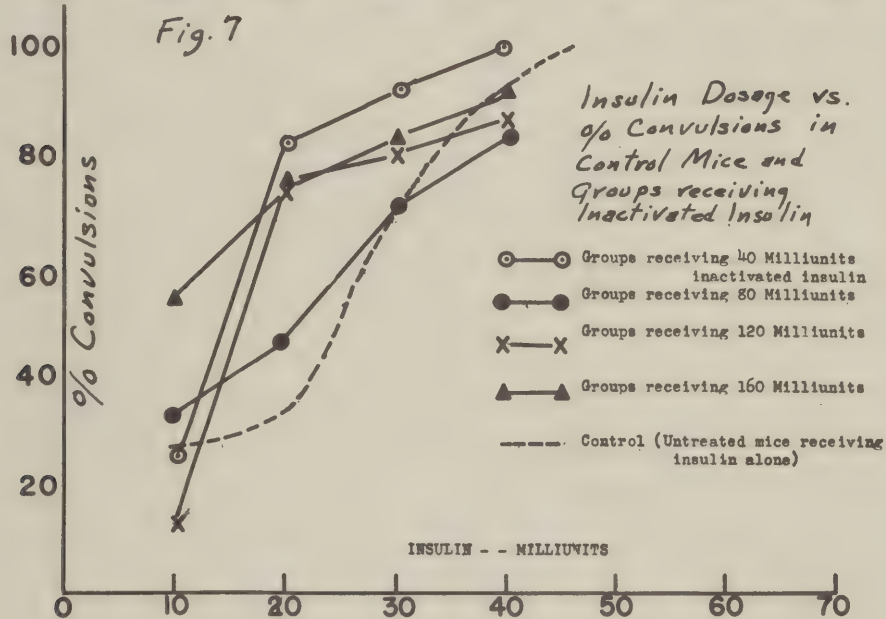
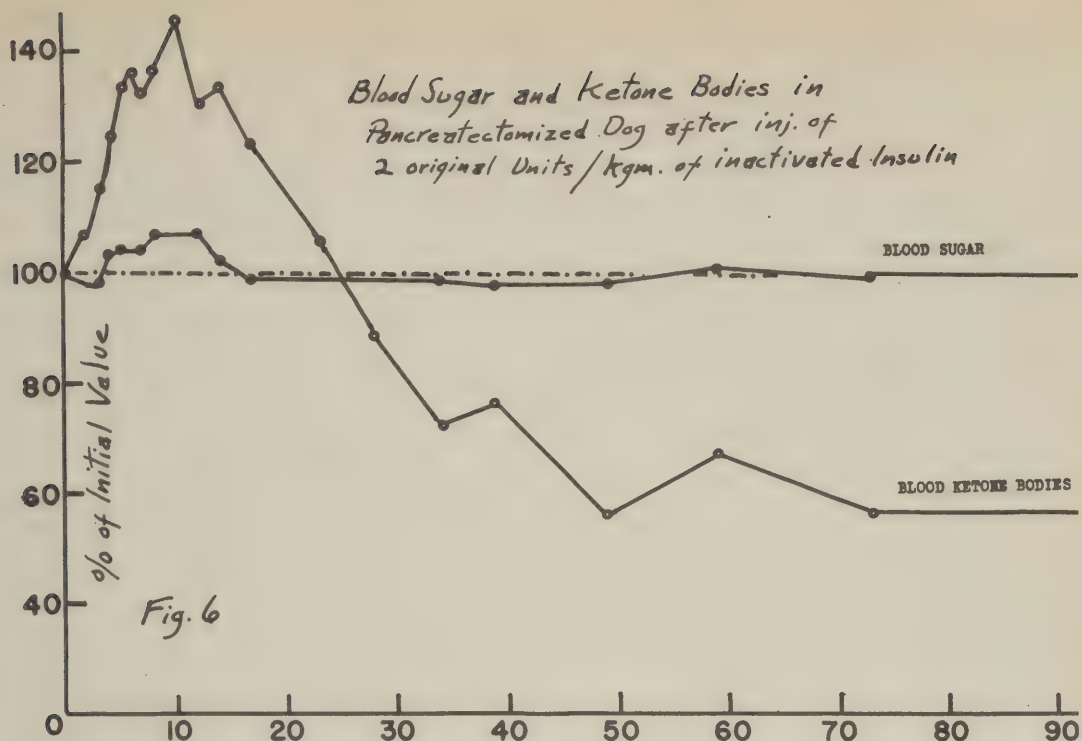
1. When the hypoglycemic activity of insulin is removed by chemical inactivation, a consistent hyperglycemic action remains. This phenomenon, originally demonstrated by Bürger, has been substantiated by reduction-inactivation using cysteine. Since similar results were obtained with "regular" insulin and zinc-insulin crystals, it appears that the effect is not based on a contaminant peculiar to amorphous preparations.

2. Despite their hyperglycemic action, these preparations could not be shown to have any protective activity against insulin-induced convulsions in mice. This seems to indicate that the hyperglycemic principle does not interfere directly with the action of insulin, but plays an independent role in the mechanisms of blood sugar regulation.

3. The hyperglycemic effect is present but diminished in uncontrolled diabetic animals and is accompanied by parallel changes in blood ketone levels. This may constitute evidence that the point of action of this principle is the hepatic glycogen stores.

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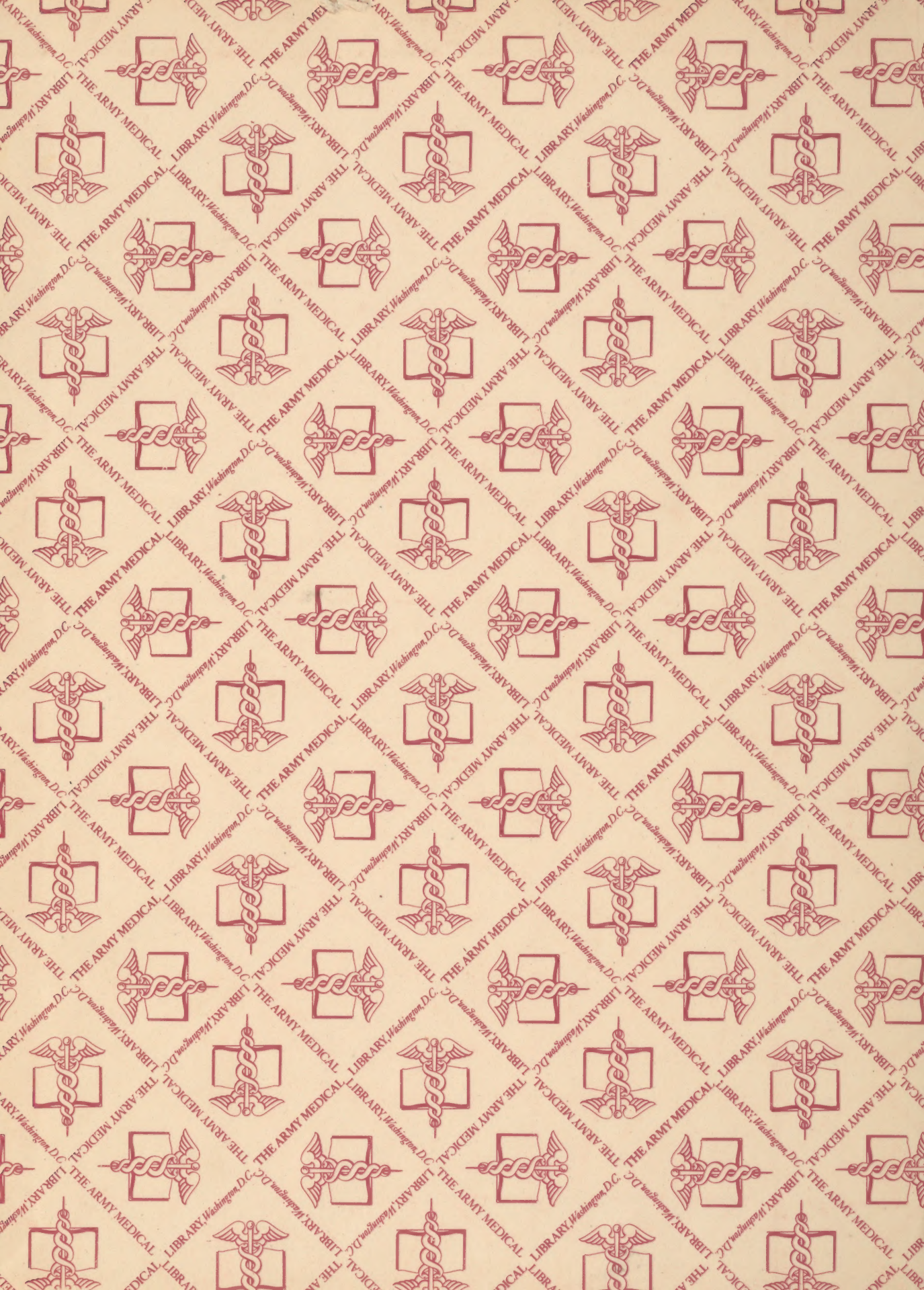




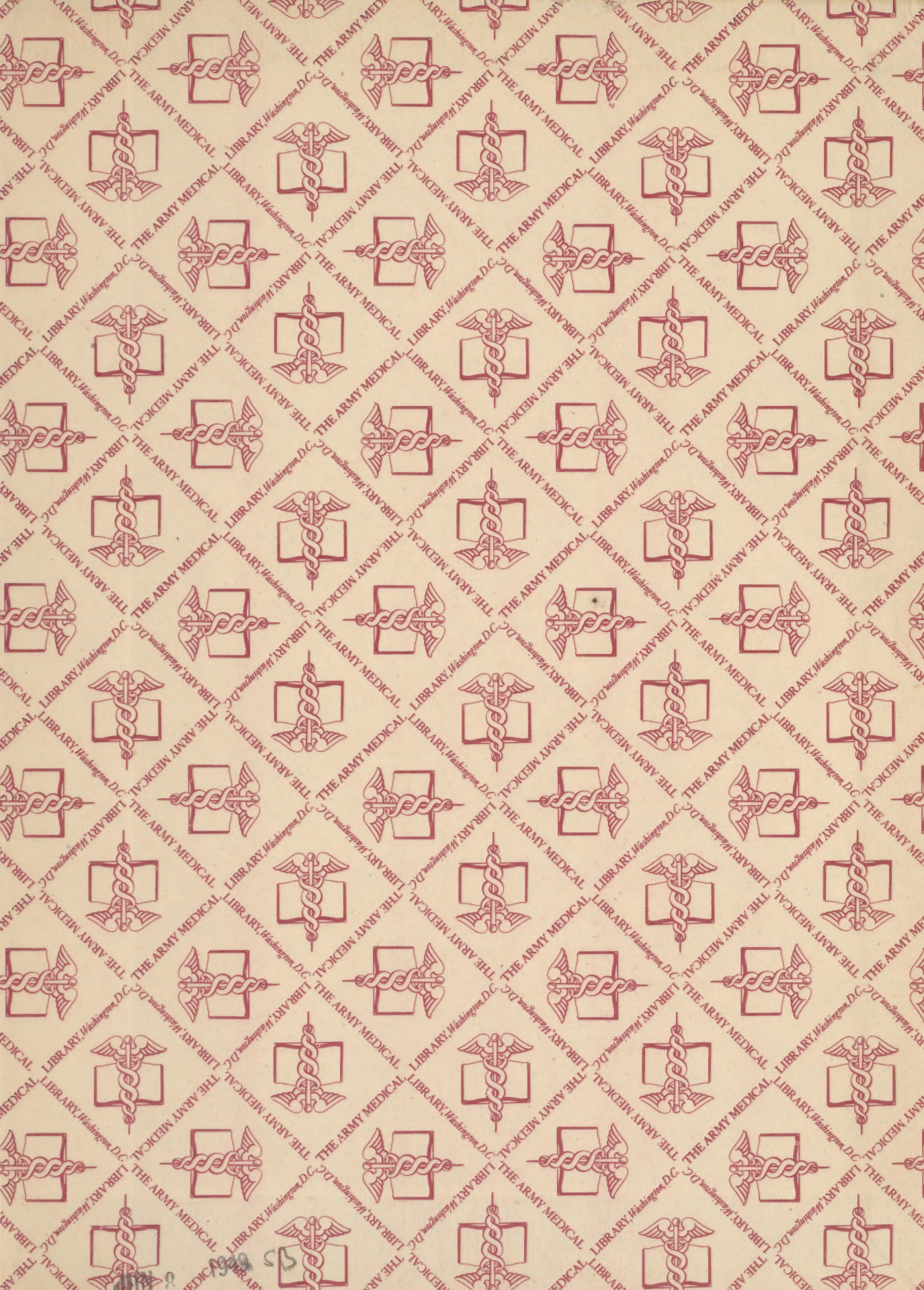














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